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EDITORIAL

Acta Oncologica Nordic Precision Cancer Medicine Symposium 2023 – merging clinical research and standard healthcare

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Introduction

Acta Oncologica has for several decades supported Nordic cancer-related symposia, and in 2023 a new biannual *Acta Oncologica* Nordic Precision Cancer Medicine Symposium (NPCM) series was initiated. The first NPCM conference 'Merging Clinical Research and Standard Healthcare' took place in Oslo, September 17–19 2023 and was hosted by Oslo University Hospital and the Norwegian Centre for Clinical Cancer Research, MATRIX. Over 2 days, the conference gathered participants from key precision medicine environments from Australia, the US, and Europe.

Precision cancer medicine is changing oncology through advanced molecular profiling, innovative clinical trials, and an increasing number of targeted drugs and treatment options. Identified molecular properties may explain why patients with the same type and stage of cancer respond differently to the same treatment. For the precision cancer medicine approach to have an impact and move towards implementation in national healthcare systems, it is essential to have access to both advanced molecular diagnostics and drugs. Although the promise of precision cancer medicine is clear and novel anti-cancer drugs targeting genetic alterations enter the market every year, implementation is still challenging. Access to these approaches is unequal due to varying availability of adequate molecular diagnostics, uncertainties regarding real-world effectiveness, hurdles regarding co-payment and reimbursement, and limited access to clinical trials and early access programmes.

Over the last decade, several national initiatives have addressed the challenges with implementation of precision cancer medicine, and during the NPCM 2023 conference, the different initiatives gathered to share and discuss key learnings and synergy potential of international collaboration within this field.

The first Nordic Precision Cancer Medicine Symposium brought together experts from different areas important for precision cancer medicine implementation into standard healthcare, and topics addressed during the conference included molecular pathology and molecular tumour boards (MTBs), biomarkers for stratification, clinical study design, DRUP-like clinical trials, scaling of precision medicine ecosystems as well as health economics, implementation, and policies. In this special edition focusing on precision medicine, altogether 10 speakers and poster presenters at the NPCM publish recent precision cancer medicine updates.

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Precision cancer medicine, molecular diagnostics, biomarkers, clinical trials, DRUP-like clinical trials, health economics

Keynote lectures: Precision cancer medicine from bench to bed

Three keynote speakers presented new developments within the precision cancer medicine field at the NPCM 2023, including presentations on cutting-edge molecular diagnostics, the Australian implementation initiative, and regulatory developments.

Gordon Mills from the Knight Cancer Institute, Oregon Health & Science University, presented a new clinical study design, targeting adaptive responses in cancer. Malignant cells and the tumour environment adapt to therapy. In the Serial Measurements of Molecular and Architectural Responses to Therapy (SMMART) trial, the patient's cancer is followed over time through serial biopsies and comprehensive analysis of tumour cells and the tumour ecosystem. Drug and drug combinations are subsequently adjusted based on these analyses to avoid resistance. A big challenge of multi-drug

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treatment is to measure adaptive responses in real-time, and tools beyond RECIST criteria are therefore required.

David Thomas from the Garvan Institute of Medical Research in Sydney gave an overview of the Australian precision cancer medicine initiatives. Omico has established comprehensive genomic profiling for patients with advanced or incurable cancer. The national Molecular Screening and Therapeutics study enrols patients with incurable cancer and has so far recruited 750 patients, and the new PrOSPeCT programme is a precision oncology screening platform enabling clinical trials by linking genomic technology to trials of new therapeutic products. Thus, Australian patients with advanced cancer have access to systematic precision cancer medicine.

Francesco Pignatti from the European Medicines Agency presented pan-cancer drug development from a regulatory perspective. Pignatti addressed some of the challenges with tumour-independent indications and approving drugs based on single-armed trials. Pignatti concluded that approval of biomarker-driven indications is similar to other approvals in high-unmet need situations. Moreover, the importance of addressing knowledge gaps prior to an approval process was emphasized.

Conference Sessions: Sharing experiences and highlighting collaboration for implementation of precision cancer medicine

The NPCM conference consisted of five conference sessions addressing molecular precision diagnostics and MTBs, design of clinical trials, health economics, implementation and guidelines, scaling of precision medicine ecosystems, and the growing ecosystem of DRUP-like clinical trials. In each session, three internationally invited speakers presented front-line research connected to the topic. In addition, short talks selected from abstract submissions were included.

Session one, Molecular pathology and MTBs, addressed advanced precision diagnostics. Access to adequate molecular profiling is crucial for the success of precision medicine. The three invited speakers in this session, Funda Meric-Bernstam from the MD Anderson Cancer Centre in Houston, Texas, Maud Kamal from Institut Curie in Paris, and Lynette Sholl from the Bringham and Women's Hospital and Harvard Medical School in Boston, highlighted key learnings from ongoing initiatives. Meric-Bernstam emphasized that a comprehensive analysis on DNA/RNA/protein is necessary to improve patient selection and treatment planning. Kamal gave an overview from their MTB and highlighted the need for clinical practice guidelines in genomic testing as well as the need to provide decision support tools and train physicians to interpret genomic data. Sholl gave an overview of the institutional cancer profiling in Boston, where more than 45,000 patients have already been screened. She addressed that 10–20% of cancer patients harbour a germline alteration conferring cancer susceptibility, and that testing for tumour-only misses important germline variants. A paired tumour-germline testing platform has therefore been established and implemented in Boston. Sholl emphasized that operationalising routine germline testing for cancer patients requires substantial inter-disciplinary teamwork. In this *Acta Oncologica* special edition, two NPCM short talk speakers present new findings highlighting the importance of risk stratification and molecular profiling. The Seibert lab in San Diego addresses risk stratification in prostate cancer screening [1]. Niehusmann et al. focus on molecular profiling and inclusion of CNS-tumour patients in the national IMPRESS-Norway trial, and the paper presents work related to precision diagnostics and therapeutic implications in desmoplastic non-infantile ganglioglioma [2]. Moreover, Fjørtoft et al. in this special issue present a review focusing on the immune microenvironment upon breast cancer progression [3]. Increased understanding of disease mechanisms is important to continue to develop the precision cancer medicine field moving forward.

Session two focused on the need for innovative clinical study designs in the field of precision cancer medicine. Richard Schilsky from the University of Chicago presented the Targeted Agent and Profiling Utilization Registry (TAPUR) study [4], the planning of which inspired several of the European national initiatives, including the DRUP trial in the Netherlands. TAPUR is a pragmatic, multi-basket, non-randomized trial where targeted FDA (U.S. Food & Drug Administration) approved drugs are used outside indication. Results from TAPUR show that 34 cohorts have been completed [5]. Emile Voest from the DRUP study [6] highlighted how a network of DRUP-like clinical trials across Europe collaborate to share data and combine cohorts across trials, greatly enhancing the impact of the individual national initiatives. In this *Acta Oncologica* special issue, these large European consortia and their impact are described in more detail [7]. Furthermore, there is still a need for new innovative clinical trial designs as highlighted by Christophe Le Tourneau from the Institut Curie in Paris, and Voest also presented the novel DRUP ATTAC study design, offering combinatorial treatment in the presence of multiple molecular targets.

Session three addressed how precision cancer medicine challenges established models for reimbursement, and there is, thus, a need for policy invasion to facilitate implementation of precision oncology. Sahar B. van Waalwijk van Doorn-Khosrovani from the National Funder's Committee for Evaluation of Specialised Medicines and Companion Diagnostics, CZ Health Insurance, The Netherlands explained how the risk-sharing reimbursement model in the DRUP and DAP (Drug Access Protocol) studies addresses the challenges when reimbursement decisions are made based on single-arm trials. The risk-sharing reimbursement models handle uncertainties regarding evidence and costs to maintain the sustainability of the healthcare system. Katarina Steen Carlsson from the Swedish Institute for Health Economics reflected on how existing Health Technology Assessment (HTA) models can be adapted to facilitate reimbursement decisions in precision cancer medicine. Moreover, Bettina Ryll from the Stockholm School of Economics Institute for Research described how a national multi-stakeholder ecosystem is necessary for precision cancer medicine implementation. She also highlighted how the European DRUP-like trial community is a self-organizing open

innovation ecosystem interacting with national decision-makers, payers, HTA, commercial sector, and civil society [7]. Monika Frenzel from the French National Research Agency described the European funding programmes for personalised medicine. In particular, Frenzel presented the European Partnership for Personalised Medicine (EP PerMed) programme that was launched towards the end of 2023. This strategic platform will run for 10 years with an approximate budget of 330 million Euros.

Session four focused on scaling of precision medicine ecosystems. Technology scaling is a major challenge when broadening precision cancer medicine initiatives to a national level. Jesus Garcia-Foncillas from the Jiménez Diaz Foundation University Hospital in Madrid and Benedikt Westphalen from the Munich Comprehensive Cancer Center shared their experiences in the rapidly evolving precision cancer medicine landscape. Kadri Toome from Tartu University Hospital, presented the results from the Estonian initiative where the National Health Insurance Fund is financing tumour profiling at a national level. Estonia is currently in the process of establishing a DRUP-like clinical trial, EstOPreT [7].

The final session on the growing ecosystem of DRUP-like clinical trials and the European-wide initiatives PCM4EU and PRIME-ROSE [7], included updates from all ongoing DRUP-like clinical trials in Europe. Hans Gelderblom from Leiden University Medical Center presented the original DRUP trial [6]. The trial opened in 2016 and key elements to the DRUP success include MTBs, good research infrastructures, and involvement of payers and pharmaceutical companies. The latest update from the DRUP trial is presented by Mohammad et al. in this special edition [8]. Moreover, Gelderblom described how the first stage three expansion cohort using nivolumab for treatment of dMMR/MSI solid tumours met evaluation criteria, resulting in reimbursement of this treatment since July 2022 in the Netherlands. The second stage three cohort includes olaparib treatment of patients with BRCA mutated tumours. This cohort will include patients from several DRUP-like clinical trials. Åslaug Helland from Oslo University Hospital gave an update from the IMPRESS-Norway trial [9, 10]. The trial started accrual in April 2021 and has so far included 1167 patients in the molecular profiling phase. Of these, 31% had an actionable molecular alteration and a matching targeted drug eligible for inclusion in the treatment phase of the study [10]. According to Puco et al., 40% of the treated patients showed clinical benefit at 16 weeks [10]. IMPRESS-Norway has started recruitment of patients with biallelic BRCA1/2 inactivation to the stage three olaparib cohort, which is financed through public–private risk-sharing modelled after DRUP. Kristoffer Rohrberg from the Copenhagen University Hospital presented the ProTarget trial [11], which has been running for 3 years. ProTarget has so far evaluated 5000 genomic profiles and 185 patients have been treated in 112 cohorts. Katriina Jalkanen from the Helsinki University Hospital presented the FINPROVE trial at the conference, and an update is also published in this special issue [12]. The trial opened at the end of 2021, and so far, 310 patients have been evaluated and 85 patients have been offered treatment. Loic Verlingue from Centre Leon Berard in Lyon gave an overview of the multi-centric MOST trials MostPlus and MEGAMOST, with altogether 14 cohorts. MostPlus has so far treated 145 patients, and the latest update from the MOST trial family is presented in this precision cancer medicine edition [13]. The DETERMINE trial in the UK was presented by Matthew Krebs from the University of Manchester. This trial opened in November 2022 and is recruiting via existing national screening programmes. This Acta Oncology special edition presents two additional precision cancer medicine initiatives in Portugal [14] and Hungary [15], respectively. The recently opened Precision Oncology Platform (POP) trial is pioneering the implementation of a precision cancer medicine strategy in Portugal [14]. Toth et al. describe the application of comprehensive molecular genetic profiling in precision cancer medicine in Hungary [15], which is the first crucial infrastructure that needs to be in place for successful precision cancer medicine implementation. Altogether, there are several well-established national initiatives, and some of these are described in detail in this special issue. Kjetil Taskén from the Oslo University Hospital rounded off the NPCM conference with an overview of how the DRUP-like clinical trial communities collaborate through the EU-funded initiatives PCM4EU and PRIME-ROSE as also described in this issue [7].

Conclusion

This first *ACTA Oncologica* Nordic Precision Cancer Medicine Symposium gathered renowned speakers from all over the world and facilitated increased international collaboration. The talks sparked good discussions and a vibrant and interactive environment. The next conference is planned for 2025. In this *Acta Oncologica* special issue, some of the addressed topics and relevant updates are described in more detail.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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SHORT REPORT

PCM4EU and PRIME-ROSE: Collaboration for implementation of precision cancer medicine in Europe

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ABSTRACT

Background: In the two European Union (EU)-funded projects, PCM4EU (Personalized Cancer Medicine for all EU citizens) and PRIME-ROSE (Precision Cancer Medicine Repurposing System Using Pragmatic Clinical Trials), we aim to facilitate implementation of precision cancer medicine (PCM) in Europe by leveraging the experience from ongoing national initiatives that have already been particularly successful.

Patients and methods: PCM4EU and PRIME-ROSE gather 17 and 24 partners, respectively, from 19 European countries. The projects are based on a network of Drug Rediscovery Protocol (DRUP)-like clinical trials that are currently ongoing or soon to start in 11 different countries, and with more trials expected to be established soon. The main aims of both the projects are to improve implementation pathways from molecular diagnostics to treatment, and reimbursement of diagnostics and tumour-tailored therapies to provide examples of best practices for PCM in Europe.

Results: PCM4EU and PRIME-ROSE were launched in January and July 2023, respectively. Educational materials, including a podcast series, are already available from the PCM4EU website (http://www.pcm4eu.eu). The first reports, including an overview of requirements for the reimbursement systems in participating countries and a guide on patient involvement, are expected to be published in 2024.

Conclusion: European collaboration can facilitate the implementation of PCM and thereby provide affordable and equitable access to precision diagnostics and matched therapies for more patients.

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Introduction

In recent years, evidence on the role of multigene sequencing in improving outcomes in patients with metastatic cancer has been widened, reinforcing the importance of precision medicine [1–3]. Several precision cancer medicine (PCM) initiatives have demonstrated the feasibility and benefits of implementing PCM within individual countries. PCM4EU (Personalized Cancer Medicine for all EU citizens) (http://www.pcm4eu.eu/) and PRIME-ROSE (Precision Cancer Medicine Repurposing System Using Pragmatic Clinical Trials) (http://www.prime-rose.eu/), are two projects funded through the EU4Health and Horizon Europe's EU Mission on Cancer programmes. The projects are built on the successes of national initiatives centred around Drug Rediscovery Protocol (DRUP)-like clinical trials (DLCTs), intending to expand equitable and sustainable access to PCM for more patients by addressing key challenges related to implementation (see Figure 1).

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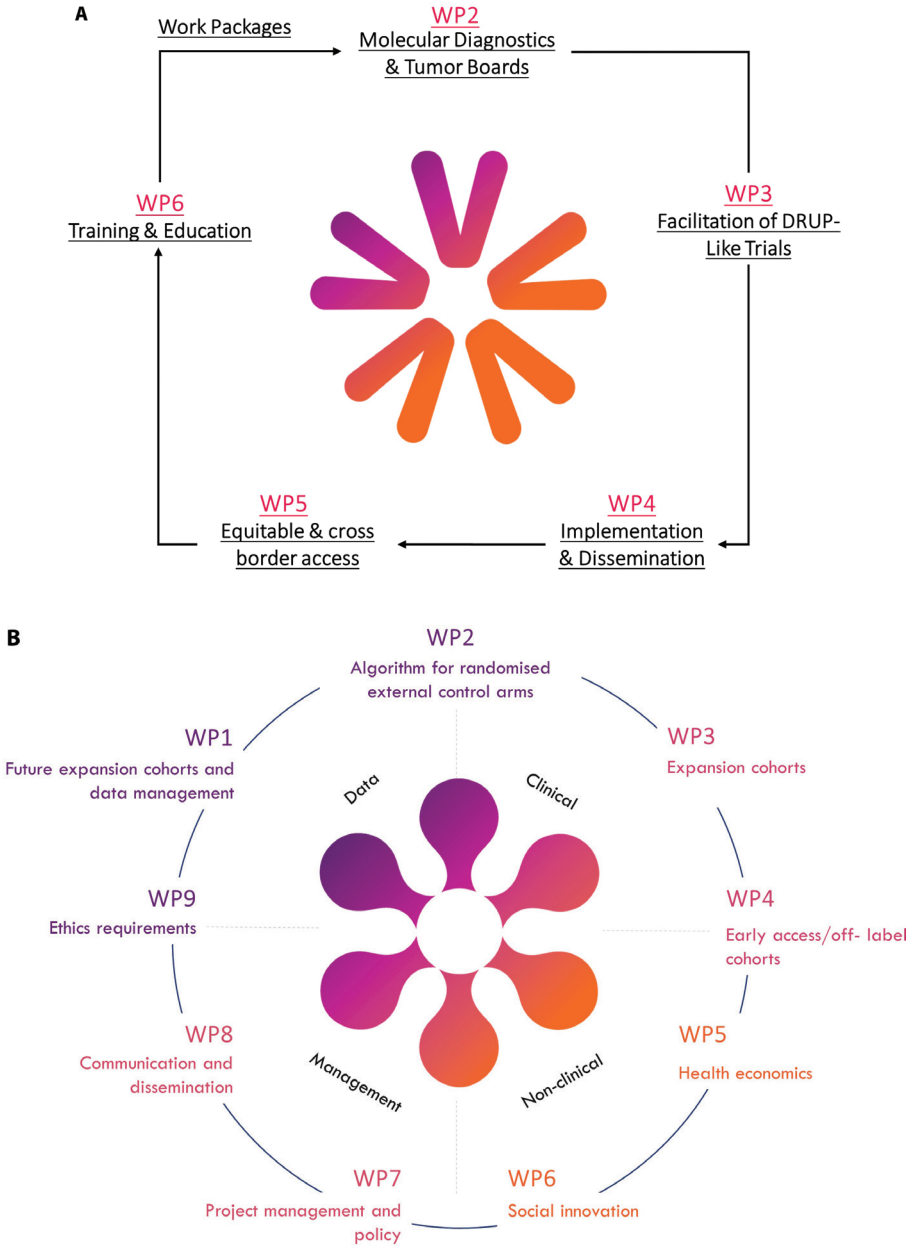

Figure 1. Overview of the planned work-packages. (A) Work packages in PCM4EU. H. Gelderblom (PCM4EU coordinator) is leading WP1, A. Edsjö and H. Russnes are leading WP2, U. Lassen and Å. Helland are leading WP3, E. Hult and K. Taskén are leading WP4, B. Ryll and U. Lassen are leading WP5, and I. Lugowska and J.Y. Blay are leading WP6. (B) Work packages in PRIME-ROSE. H. Gelderblom is leading WP1, R. Falk is leading WP2, Å. Helland is leading WP3, S.B van Waalwijk van Doorn-Khosrovani is leading WP4, K. Carlsson is leading WP5, B. Ryll is leading WP6, K. Taskén (PRIME-ROSE coordinator) is leading WP7, K. Jalkanen is leading WP8 whereas Charlotte J. Haug is the independent ethics review in WP9.

The success of the original DRUP-trial from the Netherlands, showing high inclusion rate and clinical benefit (CB), inspired similar trials in other countries [4]. All involved national trials are based on or aligned with DRUP (PIs Emile Voest, Hans Gelderblom, Henk Verheul) [5, 6], while being independently organised, governed and financed (Table 1). Such trials are ongoing or soon to be initiated in 11 European countries (Figure 2A), with more countries preparing to launch DLCTs. DRUP is also collaborating with the American TAPUR Study and CAPTUR in Canada, that are built on the same principles as the European trials [7, 8], and has aggregated data with the Australian MoST trial [9].

DLCTs are prospective phase II combined umbrella-basket trials in which patients with advanced cancers receive targeted therapies matched to genomic alterations in their tumour. Each trial enrols participants into defined cohorts, based on the combination of tumour type, molecular alteration, and targeted therapy, with CB as the primary endpoint. Common for the DLCTs is that they combine accessibility with affordability by providing broad access to PCM for patients who have exhausted all other standard treatment options. The trials continuously generate evidence that can be linked to pragmatic outcome-based reimbursement schemes, thereby enabling reimbursement when the necessary evidence (e.g. the PASKWIL

Table 1. Overview of overlapping primary and secondary endpoints in the DRUP-like clinical trials.

Endpoint in DRUP-like clinical trials	DRUP	ProTarget	IMPRESS	FINPROVE	MOSTplus	DETERMINE	POP	FOCUSE	ESTOPRET
Disease control at 16 weeks after treatment initiation	X	X	X	X	X	X*	X	X	X
Progression-free and overall survival	X	X	X	X	X	X	X	X	X
Duration of treatment on study (time on drug)	X	X	X	X	X	X	X	X	X
Treatment related grade ≥3 and SAE	X	X	X	X		X	X	X	X
Objective tumour response	**X**	**X**	**X**		**X**	**X**	**X**	**X**	
% of patients treated based on their molecular profile	**X**	**X**	**X**		**X**		**X**	**X**	**X**

An overview of overlapping main endpoints in the ongoing DRUP-like clinical trials. *Durable Clinical Benefit, defined as the absence of disease progression for at least 24 weeks from the start of trial treatment. Dark blue = 100% similarity across trials, blue = 90% similarity across trials, and light blue = 80% similarity across trials.

criteria in the Netherlands) has been gathered [10, 11]. An overview of on-label and off-label reimbursement systems in Europe is also part of the deliverables in PRIME-ROSE.

The projects will provide recommendations regarding the use of next-generation sequencing (NGS)-panels and clinical decision support system (CDSS)-tools, together with detailed guidelines for molecular diagnostics in the cancer care pathway to facilitate implementation of PCM in additional countries. Furthermore, novel methods for establishing relative effectiveness, as well as strategies for evaluation of cost-effectiveness of PCM, will be developed. This will be used to facilitate access to new treatment options.

Advancing Molecular Diagnostics in PCM

PCM4EU aims to facilitate implementation of adequate molecular diagnostics into standard-of-care for all patients in Europe (Figure 1A). Clinical molecular diagnostic assays must detect all relevant genetic variants for adequate therapy decisions, while simultaneously identifying patients for clinical trials. The rapid development of new classes of targeted therapies and the promising results of immunotherapy, both necessitating use of more complex biomarkers, has resulted in a growing need for tools to guide in choosing NGS-based assays and CDSS tools.

To address this challenge, PCM4EU is gathering data on NGS-based assays used by the participating centres to create a curated database containing information on gene panel content in relation to targeted therapy and clinical trial inclusion criteria. Several of the DLCTs use both NGS and whole genome sequencing (WGS) as part of the diagnostic work-up. Based on available data, *in-silico* comparisons will be performed on commonly used NGS-panels versus WGS, and the added value of introducing WGS will be evaluated through health technology assessment. Real-life and synthetic large-scale datasets will be developed to harmonise the interpretation of key complex biomarkers such as microsatellite instability (MSI), tumour mutational burden (TMB), and homologous recombination deficiency (HRD).

PCM4EU will map out currently available CDSS tools for clinical decision-making in oncology. Evaluation will include feature mapping, tool requirements, integration options, compatibility with requirements for patient security, and *In Vitro* Diagnostic Regulation status. To expand and structure knowledge on available CDSS tools, including artificial intelligence-based tools, these tools and their knowledge management strategies will be charted. Moreover, the project will develop a conceptual framework for performance testing across multiple cancer-relevant features beyond the core variant characterisation, like single nucleotide variants and insertions and deletions, including copy-number variations, selected complex biomarkers (i.e., TMB, HRD, MSI), neoantigens, transcription profiles and specific gene signatures (e.g. immune signatures), and gene fusions. Finally, we plan to evaluate the performance of clinical trial matching across CDSS platforms to improve harmonisation between different recommendations by enhancing the accuracy of available clinical trial data and mapping the identification and definition of actionable targets [12].

Initiation of DLCTs in Europe

The PCM4EU and PRIME-ROSE projects will support countries without DLCTs in setting up trials in countries where they are not yet available by sharing current protocols, standard operating procedures, electronic case report forms (eCRFs), and ways for harmonised data collection (Figure 1). Each DRUP-founder country will co-create efficient knowledge transfer to centres in the start-up phase.

For joint data analyses across DLCTs, the key primary endpoint for the combined analyses is uniformly implemented across the trials and defined as CB, meaning a confirmed stable disease, partial or complete response at 16 weeks after treatment initiation (Table 1). A formal data sharing protocol has been developed and agreed upon.

Access to New Therapies: Single Point Access to Multi-Trial Network

Data sharing and aggregation between the trials will allow for monitoring of cohort recruitment from each trial and cross-trial evaluation of cohorts when recruitment is complete, increasing the inclusion rate. Moreover, data sharing will facilitate collaboration with the pharmaceutical industry. Pharmaceutical companies can choose to access all trials simultaneously with their drugs through PRIME-ROSE, providing a single point of entry, where pharma partners provide free drugs and floating treatment slots to the consortium. This approach will significantly reduce time for initiation and rapidly increase inclusion rates

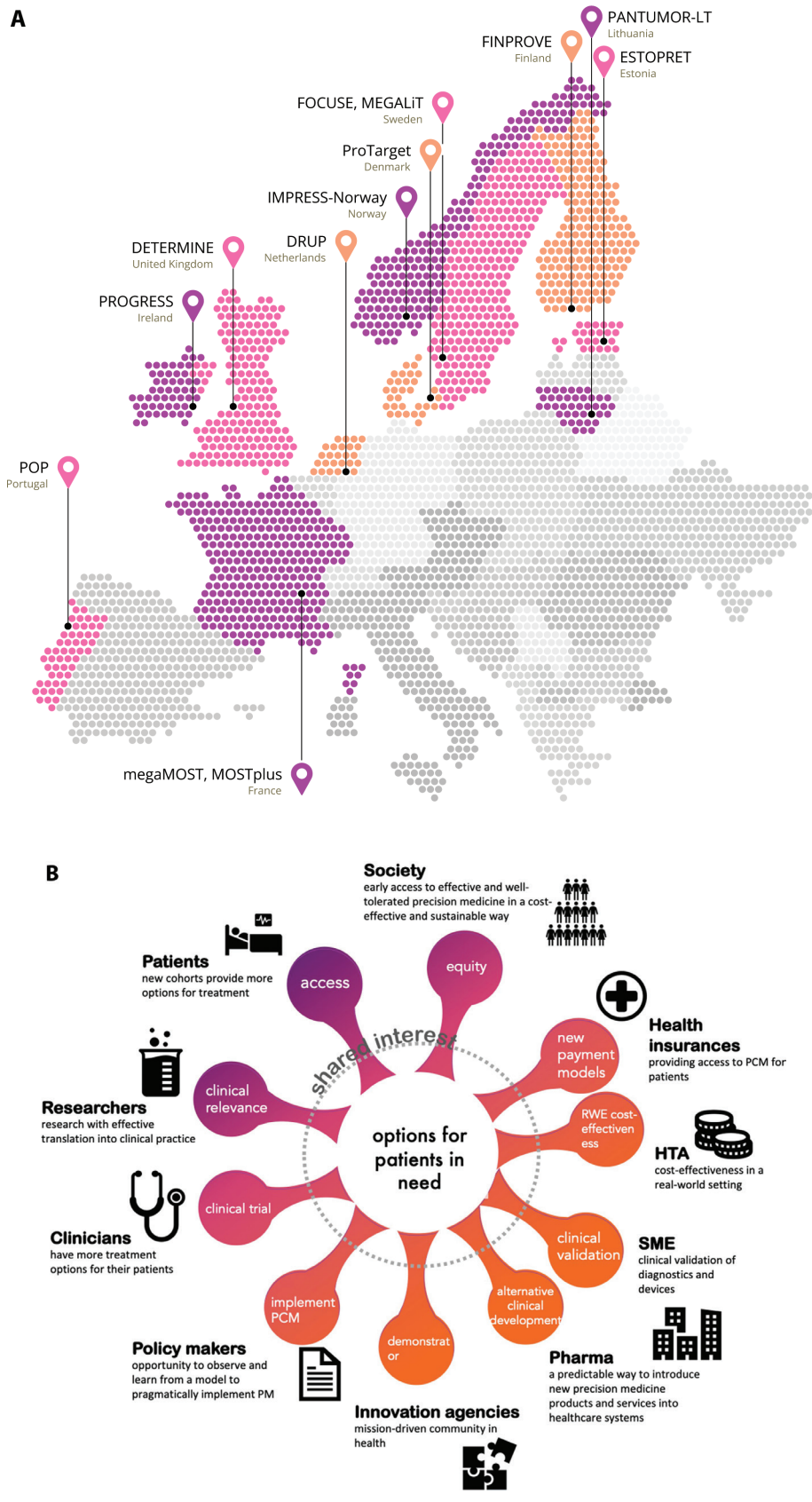

Figure 2. Overview of all ongoing and soon-to-start DRUP-like trials in Europe. (A) DRUP in the Netherlands, PIs E.E Voest, H. Gelderblom & H.M.W. Verheul, ProTarget in Denmark, PIs K.S. Rohrberg & U. Lassen. FINPROVE in Finland, PI K. Jalkanen, IMPRESS-Norway in Norway, PI Å. Helland, MOSTplus and mega-MOST in France, PIs J.Y. Blay & L. Verlingue, DETERMINE in United Kingdom, PI M.G. Krebs, POP in Portugal, PI J. Oliveira, ESTOPRET in Estonia, PI K. Ojamaa, and PANTUMOR-LT in Lithuania, PI E. Baltruškevičienė. (B) Stakeholder involvement in PRIME-ROSE.

while removing a major barrier for establishing a DLCT, since access to drugs is critical for trial initiation.

Establishment of Relative Effect: Innovative Models for Synthetic Control Cohorts

DLCTs are actively searching for patients with defined target mutations to treat them with the specified drugs. Randomisation for treatment allocation after progression on standard-of-care is not advised due to the rarity of driver genomic alterations and the lack of treatment alternatives. To establish relative efficacy, multiple synthetic control cohorts will be built to reduce bias and resembling a randomised controlled trial.

PRIME-ROSE will establish three types of control cohorts, based on available data from registries and sequenced cohorts that have received standard-of-care therapy: 1) Patients with a known genomic alteration that received standard treatment. However, as specific molecular alterations will have a low prevalence, these control cohorts might be small. 2) Patients without a targetable genomic alteration. The characteristics of patients with and without genomic alterations will be compared to find patterns associated with specific targets and calculate the expected probability of the alterations. 3) Extraction of larger patient control cohorts from registry data. For these cohorts, information about the presence or absence of a specific mutation will not necessarily be available, but the frequency of the mutation in the population may be known. Therefore, based on the probability of the target mutation, randomisation will be performed to synthetic control arms to reduce selection bias.

Implementation into Standard-of-Care

Whereas regulatory approval is centralised to the European Medicines Agency, reimbursement systems are country-specific, with varying requirements in terms of data. To facilitate implementation, PRIME-ROSE will identify requirements of reimbursement systems in participating countries, including systems for off-label reimbursement. A model for economic evaluation will be designed and constructed, including budget impact and cost-effectiveness analysis of PCM, integrating the chain of decision from molecular diagnostics to treatment. Information collected on local requirements for reimbursement assessment will be used to ensure that conducted analyses will meet the criteria for decision-making in different countries. Furthermore, collected data will provide a background to evaluate and compare how differences in reimbursement systems can impact access, timing and affordability.

National Guidelines for Precision Diagnostics

To facilitate the development of national guidelines for precision diagnostics, the PCM4EU consortium will develop a best practice on which cancer patients should be offered molecular diagnostics and what should be included in the molecular diagnostic work-up as part of standard-of-care. This includes recommendations on selecting the most appropriate molecular assays and how to process data to match results with anti-cancer therapies according to up-to-date evidence (in accordance with European Society for Medical Oncology (ESMO) clinical guidelines [13]). The guidelines will focus on the added value of using advanced diagnostic tests in terms of higher precision in choosing treatment strategies as well as costs and value of implementation. We aim to include costs from a wide societal perspective to demonstrate broader impact. However, results will be reported disaggregated to allow for interpretations from more narrow perspectives preferred in some countries.

Risk-Sharing Agreement and Access to Therapies

Participants in pivotal trials are usually not representative of patients encountered in standard clinical practice, especially since rare tumour types are often not represented. Here, we face the ragged edges of clinical practice, where off-label use is common since there are no alternative treatment options, the effectiveness and safety data are often not collected, the practice is not harmonise or regulated, and there are disparities and inequalities in access. A comprehensive approach to this clinical reality can stimulate repurposing drugs to offer safe, effective, and affordable treatment options for the diverse cancer patient population commonly seen in clinical practice.

To evaluate the effectiveness of a treatment in routine clinical practice, real-world data will be systematically collected. Such practice is carried out by the DRUG Access Protocol (DAP) in the Netherlands [14]. DAP is a pragmatic, non-randomised protocol that prospectively collects effectiveness and toxicity data on novel authorised or unauthorised anticancer therapies awaiting regulatory approval and reimbursement, but also authorised anticancer therapies that are not being reimbursed for an on-label or off-label indication due to data gaps.

In PRIME-ROSE, strategies for pragmatic outcome-based risk-sharing agreements will be explored by identifying factors that were critical for the successful establishment of such agreements in the Netherlands and Norway [15]. Based on a mapping of the varying requirements as regards to the implementation of new treatments and expansion to new treatment groups, PRIME-ROSE will assist stakeholders, including national healthcare providers, policymakers, and authorities in EU regions, Member States, and Associated Countries who desire to implement pragmatic reimbursement models for PCM in a real-world setting. The potential for a European framework to evaluate data effectively and efficiently to implement PCM will also be investigated, especially for rare cancers and those with high unmet medical needs. In addition, we will explore the potential of using DRUP-generated evidence to inform regulatory decision-makers at the European level.

PCM addressing Patient Needs

The patient relevance of the clinical trial endpoints in use will be confirmed to ensure that DLCTs adequately address patients' needs. Currently, a multi-stakeholder consultation that will

inform the selection of health-related quality-of-life endpoints for use across the joint cohorts is in preparation. Furthermore, the stability of patient preferences during and after trial participation will be investigated. Moreover, we are working with a growing community of European cancer patient advocates with expertise in PCM and iterate on existing patient involvement to ensure consistent and meaningful patient involvement throughout the work of the PCM community.

Successful Multistakeholder Collaborations for Change in Complex Systems

Changes in the healthcare system require participation and interaction between a multitude of stakeholders (Figure 2B). In PRIME-ROSE, we plan to map and describe the PCM ecosystem and develop a PCM theory of change for healthcare as a complex adaptive system with implications for governance, policy, and innovation. We will reflect on differences between the Member States. Building on the concept of Living Labs [16] and the already established practice of peer-to-peer support, the project will develop a 'DRUP methodology' to facilitate implementation.

Conclusion

The PCM4EU and PRIME-ROSE projects strive to reduce inequality in cancer treatment by promoting access to PCM for all European patients through collaborative efforts, patient engagement, and pragmatic outcomes-based approaches.

Author contributions: Project design: KT, GLF, RSF, ÅH, SBWDK, KSC, BR, KJ, AE, HGR, UL, EHH, IL, JYB, LV, MAL, MGK, KSR, KO, JO, HV, EV, HG; draft manuscript: KT, GLF, SFHM, HG. All authors reviewed the results and approved the final version of the manuscript.

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Acknowledgement: Key participants in the PCM4EU and PRIME-ROSE consortia are named as consortia authors. We are also grateful to a number of individuals, institutions, industry partners, and grant agencies that are not listed, but contribute to building national initiatives and DRUP-like clinical trials in each country.

Disclosure statement: The authors report there are no competing interests to declare.

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Data availability statement

No new data were presented in this study. Data sharing is not applicable to this short report.

Ethics declarations & trial registry information

The projects reported in this manuscript adhere to high ethical standards and complies with all relevant legislation.

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SHORT REPORT

The evolution of precision oncology: The ongoing impact of the Drug Rediscovery Protocol (DRUP)

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ABSTRACT

Background and purpose: The Drug Rediscovery Protocol (DRUP) is a Dutch, pan-cancer, nonrandomized clinical trial that aims to investigate the efficacy and safety of targeted and immunotherapies outside their registered indication in patients with advanced or metastatic cancer.

Patients: Patients with advanced or metastatic cancer are eligible when there are no standard of care treatment options left and the tumor possesses a molecular genomic variant for which commercially available anticancer treatment is accessible off-label in DRUP. Clinical benefit is the study's primary endpoint, characterized by a confirmed objective response or stable disease after at least 16 weeks of treatment.

Results: More than 2,500 patients have undergone evaluation, of which over 1,500 have started treatment in DRUP. The overall clinical benefit rate (CBR) remains 33%. The nivolumab cohort for patients with microsatellite instable metastatic tumors proved highly successful with a CBR of 63%, while palbociclib or ribociclib in patients with tumors harboring CDK4/6 pathway alterations showed limited efficacy, with a CBR of 15%. The formation of two European initiatives (PCM4EU and PRIME-ROSE) strives to accelerate implementation and enhance data collection to broaden equitable access to anticancer treatments and gather more evidence.

Conclusion: DRUP persists in improving patients access to off-label targeted or immunotherapy in the Netherlands and beyond. The expansion of DRUP-like clinical trials across Europe provides countless opportunities for broadening the horizon of precision oncology.

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Targeted therapy; immunotherapy; whole-genome sequencing; precision medicine

Introduction

With the rapidly evolving field of precision oncology, the Dutch Center for Personalized Cancer Treatment (CPCT) was established a decade ago to construct an integrated infrastructure for the collection of molecular genetics data linked to clinical outcome. This facilitated the creation of a database with information on patients with metastatic cancer to enhance the landscape of predictive and prognostic biomarkers in oncology [1]. By building this infrastructure, a network of 49 collaborating hospitals throughout the Netherlands was inaugurated. Concurrently, the nonprofit organization Hartwig Medical Foundation was instituted with the purpose of conducting whole-genome sequencing (WGS) and maintaining the database [2]. Priestley et al. conducted a large WGS analysis on this database, containing 2,520 genomic tumor landscapes [3]. They concluded that 62% of patients harbored at least one actionable target, for half of which an approved anticancer treatment was available. However, in 13% of patients, the possible treatment was outside its registered indication, suggesting that these patients would not have access to potentially beneficial treatment. To overcome this unmet need, the Drug Rediscovery Protocol (DRUP) was initiated, to provide patients the possibility of treatment with off-label therapies, with the intent to focus on broader implementation of already existing drugs, rather than developing novel treatments. This investigator-initiated study aims to investigate the efficacy and safety of commercially available anticancer treatments in patients with advanced or metastatic cancer that have no more standard of care treatment options left and provide patients access to these medications based on their tumors' molecular profile [4]. Here we present an overview of DRUP, illuminating implications for the future.

Methods

Study design

DRUP is a Dutch, ongoing, multicenter, nonrandomized, prospective, umbrella, and basket trial in which patients receive off-label treatment with commercially available targeted therapy or immunotherapy based on potentially actionable

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molecular alterations in their tumor. Parallel cohorts are designed for patient enrolment, characterized by tumor type, molecular alteration and study treatment, as well as tumor-agnostic cohorts (Figure 1) [4].

Approval for DRUP (NCT02925234) was granted by the Medical Ethics Committee at the Netherlands Cancer Institute in Amsterdam, the Netherlands, following the guidelines for Good Clinical Practice and ethical principles for medical research from the Declaration of Helsinki. The protocol and study design have been published earlier in more detail [4].

Study population

Patients are eligible for the trial if they have a progressive advanced or metastatic solid tumor, multiple myeloma, or non-Hodgkin lymphoma and have no standard treatment options left. A potentially actionable genomic variant has to be identified as part of routine molecular diagnostics. Furthermore, patients need to be adults ≥18 years of age, have an adequate performance score and organ function, and have measurable disease according to the RECIST 1.1, RANO, IMWG, or Lugano, for solid tumors, high-grade gliomas, multiple myeloma, or lymphoma, respectively, which is necessary for radiological response evaluations [5–8].

Matching treatment and assessments

The study team assesses each case submission by aiming to match a specific molecular alteration with an available therapy in DRUP. For each therapy, potentially actionable alterations were predefined according to literature [9]. A more detailed description of the matching rules has been published previously [4]. After a successful match, patients are enrolled in the study and undergo screening according to drug-specific inclusion criteria. Radiological response evaluations are performed at baseline and every 8 or 9 weeks after start of treatment, depending on the study treatment. The primary endpoints are clinical benefit (CB) and safety. CB is defined as confirmed complete or partial response or stable disease after 16 weeks of treatment. Safety is measured by the frequency of grade ≥3 treatment-related adverse events following the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Objective response rate (ORR) is defined as the percentage of patients with complete or partial response.

Pretreatment biopsies

Before treatment is initiated, a mandatory fresh frozen biopsy specimen is obtained, together with a blood sample that is solely used to assess a patient's germline DNA, and sent to Hartwig Medical Foundation where WGS and RNA sequencing is performed if the tumor biopsy consists of a certain tumor-cell percentage [10]. Patients with primary brain tumors are exempt from this procedure, similar to patients for whom WGS was performed in a nonclinical trial setting prior to study enrolment, without receiving any anticancer treatment in between, as well as patients who underwent an allogenic stem cell transplantation in the past, due to the inability to perform accurate WGS because of the mismatch between the biopsy specimen and blood sample.

Statistical analysis

A Simon-like two-stage design is used to monitor cohorts (Figure 2) [11] Stage 1 consists of eight patients, of which at least one patient needs to show CB for the cohort to stay open and extend to stage 2, where 16 additional patients are added. If then five or more patients meet the criteria for CB, the cohort is considered successful and gives opportunities for more extensive investigation. An 85% power and a one-sided alpha error

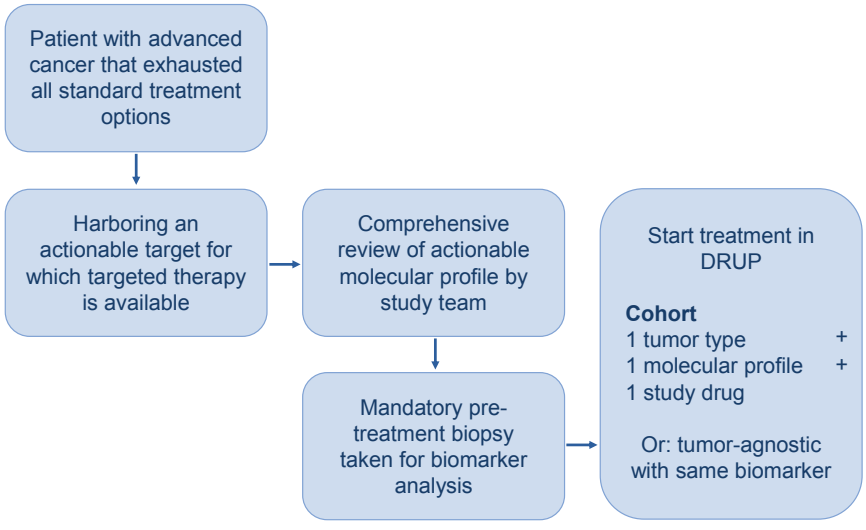

Figure 1. Study design.

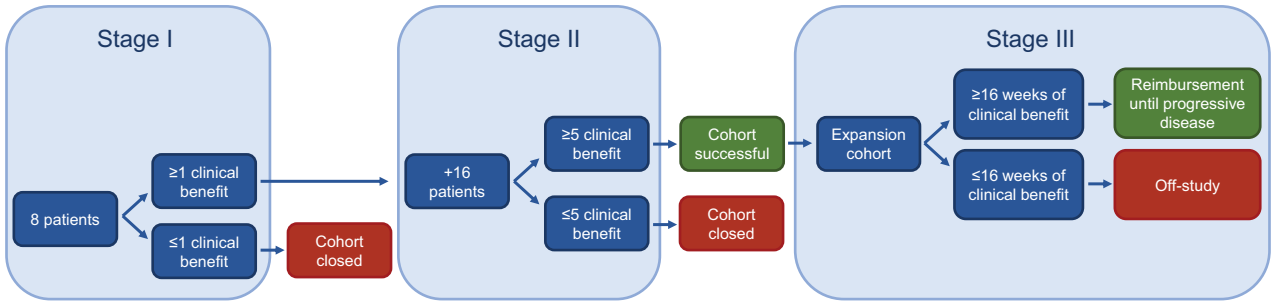

Figure 2. Cohort design.

rate of 7.8% is used to reject the null hypothesis of a 10% clinical benefit rate (CBR) if the true CBR is ≥30%, as described in earlier publications [12, 13].

Results

Since the initiation of DRUP in 2016, as of November 2023, over 2,500 patients have been submitted to the study team for potential study participation. More than 1,500 of these patients started treatment with one of the 36 available targeted- or immunotherapies in DRUP, provided by 14 different pharmaceutical companies, at one of the 35 affiliated hospitals across the country. An interim analysis of the first 500 included patients showed a CBR of 33% with similar results between rare and non-rare cancers [14].

An example of a successful stage 2 cohort is the tumor-agnostic nivolumab cohort for patients with tumors that harbor microsatellite instability (MSI), with a CBR of 63% [4]. Based on these results, a third stage cohort was opened to validate the findings from this second stage cohort in a broader population (Figure 2). This 'expansion' cohort showed promising results with a CBR of 62% and an ORR of 45% [15]. While the therapies in the first and second stage cohorts are provided free of charge by the pharmaceutical companies, a personalized reimbursement model was created for third stage cohorts to facilitate risk sharing between the pharmaceutical companies and healthcare insurers. In this model, treatment coverage is transferred to the healthcare insurers after a patient shows CB for at least 16 weeks [16]. The Dutch National Health Care Institute analyzed the positive preliminary stage 3 findings and provided a positive recommendation for the reimbursement of nivolumab for patients with pretreated advanced MSI tumors, regardless of tumor type. Consequently, these patients now have access to nivolumab outside its registered label since July 1st 2022 in the Netherlands [17]. This paves the way for more new reimbursed indications based on data from DRUP. Another promising second stage cohort is the cohort for BRCA1 and BRCA2 mutated tumors treated with olaparib. It was shown that 58% of the treated patients had CB with an ORR of 29%, therefore laying the groundwork for a possible new stage 3 cohort in the future [18]. Furthermore, patients with MET-mutated non-small cell lung cancer (NSCLC) treated with crizotinib responded extraordinarily well, with a CBR of 71% and an objective response in 62% of patients [19].

Nonetheless, it is important to note that not only successful DRUP cohorts are being published, as exemplified in a recent article by Zeverijn et al. [20]. The article reported the results of 139 patients with various tumor types, either enrolled in DRUP or the Australian Cancer Molecular Screening and Therapeutic (MoST) trial, that were treated with one of the CDK4/6 inhibitors palbociclib or ribociclib as monotherapy, based on complete loss of CDKN2A or SMARCA4 or amplifications of CDK4, CDK6, CCND1, CCND2, or CCND3. These patients exhibited limited clinical efficacy with a CBR of 15% and the absence of any objective response. Based on these findings accrual for these agents was terminated and it was concluded that palbociclib or ribociclib administered as monotherapy is not recommended.

With 218 cohorts in DRUP open for accrual at this point, it has become evident that certain combinations of molecular profiles, tumor types and treatments are very rare. Over the years, 44 cohorts have progressed to stage 2, while the remaining cohorts persist in stage 1, resulting in a median of 2 patients per cohort. While the primary goal is to provide access to potentially effective medicine to as many patients as possible, it is equally crucial to gather evidence for further implementation of these treatments. In 2021, a Memorandum of Understanding was signed between DRUP and precision oncology trials in the Nordic countries (Denmark, Norway, Sweden, and Finland), which based their protocols on the DRUP protocol to ensure alignment in trial design and endpoints, also referred to as DRUP-like clinical trials. This alignment grants the ability to share data and accelerate evidence gathering [21]. Building further on this Memorandum, two European projects were launched in 2023: Precision Cancer Medicine for all EU Citizens (PCM4EU), funded by the EU4Health program as part of Europe's Beating Cancer Plan (grant: 101079984), and Precision Cancer Medicine Repurposing System Using Pragmatic Clinical Trial (PRIME-ROSE), funded under the Horizon Europe program (grant: 101104269), aiming to broader implement DRUP-like clinical trials across the European Union. This network of independent investigator-initiated DRUP-like clinical trials focusses on data sharing to ensure equitable access to effective anticancer treatment within Europe [22].

Discussion

Since 2016, DRUP has been extending access to off-label anti-cancer treatments for patients with advanced or metastatic

cancer. This article exemplifies the ongoing impact of this precision oncology trial. Notably, the outcomes of DRUP have led to the reimbursement of nivolumab for all patients in the Netherlands with pretreated advanced tumors that harbor MSI, highlighting the potential opportunities an investigator-initiated study can provide [4, 17]. Concurrently, addressing the limited therapeutic efficacy of palbociclib or ribociclib as monotherapy for patients with alterations in the cyclin D-CDK4/6 pathway, underscores the importance of reporting negative evidence to prevent futile treatments in the future [20].

The ongoing CBR of 33% observed in DRUP demonstrates that matched targeted anticancer therapies benefit a considerable number of patients [14]. However, the remaining two-thirds of patients do not show CB to this therapeutic approach, with drug resistance standing out as a primary contributor to the unresponsiveness to treatment. Despite the extensive research on the field of resistance, intrinsic as well as acquired, conquering this hurdle continues to be challenging [23]. A better understanding of the underlying mechanisms for resistance to targeted monotherapy provides opportunities for optimized treatment possibilities, including combination therapy, together with improved patient selection, even within the framework of DRUP, underlining the importance of broad implementation of genomic testing.

Some limitations warrant consideration in the context of this study. Primarily, the diversity of the study population might challenge data interpretation. Additionally, the absence of a comparison group could complicate the interpretation of the treatment efficacy. Nevertheless, it is essential to acknowledge the ethical objections of withholding patients potentially effective treatment while they have no therapeutic options left. To overcome these challenges, novel methodologies have been suggested, incorporating the utilization of real-world data as a control mechanism [24]. This approach is being investigated within the framework of the European project PRIME-ROSE.

In conclusion, DRUP continues to provide patients with advanced or metastatic disease access to off-label targeted or immunotherapy based on their genetic tumor profile. The establishment of European collaborations of DRUP-like clinical trials holds promise for expanding the scope of precision oncology.

Author contributions

Soemeya F. Haj Mohammad: Investigation, Visualization, Project administration, Writing – Original Draft
Hans J.L. Timmer: Investigation, Project administration, Writing – Review and Editing
Laurien J. Zeverijn: Investigation, Project administration, Writing – Review and Editing
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Henk M.W. Verheul: Conceptualisation, Methodology, Writing – Review and Editing, Supervision
Emile E. Voest: Conceptualisation, Methodology, Writing – Review and Editing, Supervision
Hans Gelderblom: Conceptualisation, Methodology, Writing – Review and Editing, Supervision

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Disclosure statements

The authors report there are no competing interests to declare.

This article is based on a presentation by one of the co-authors (HG) at the Acta Oncologica sponsored Nordic Precision Cancer Medicine Meeting 17–19 September 2023 in Oslo.

Ethics declaration

Approval for DRUP (NCT02925234) was granted by the Medical Ethics Committee at the Netherlands Cancer Institute in Amsterdam, the Netherlands, following the guidelines for Good Clinical Practice and ethical principles for medical research from the Declaration of Helsinki.

Data availability statement

All data described in this study are freely available for academic use and can be obtained through a request to the corresponding author by email.

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SHORT REPORT

IMPRESS-Norway: improving public cancer care by implementing precision medicine in Norway; inclusion rates and preliminary results

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ABSTRACT

Background and purpose: In Norway, comprehensive molecular tumour profiling is implemented as part of the public healthcare system. A substantial number of tumours harbour potentially targetable molecular alterations. Therapy outcomes may improve if targeted treatments are matched with actionable genomic alterations. In the IMPRESS-Norway trial (NCT04817956), patients are treated with drugs outside the labelled indication based on their tumours molecular profile.

Patients and methods: IMPRESS-Norway is a national, prospective, non-randomised, precision cancer medicine trial, offering treatment to patients with advanced-stage disease, progressing on standard treatment. Comprehensive next-generation sequencing, TruSight Oncology 500, is used for screening. Patients with tumours harbouring molecular alterations with matched targeted therapies available in IMPRESS-Norway, are offered treatment. Currently, 24 drugs are available in the study. Primary study endpoints are percentage of patients offered treatment in the trial, and disease control rate (DCR) defined as complete or partial response or stable disease in evaluable patients at 16 weeks (W16) of treatment. Secondary endpoint presented is DCR in all treated patients.

Results: Between April 2021 and October 2023, 1,167 patients were screened, and an actionable mutation with matching drug was identified for 358 patients. By the data cut off 186 patients have initiated treatment, 170 had a minimum follow-up time of 16 weeks, and 145 also had evaluable disease. In patients with evaluable disease, the DCR was 40% (58/145). Secondary endpoint analysis of DCR in all treated patients, showed DCR of 34% (58/170).

Interpretation: Precision cancer medicine demonstrates encouraging clinical effect in a subset of patients included in the IMPRESS-Norway trial.

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Introduction

Precision medicine is changing oncology by leveraging advanced molecular precision diagnostics, innovative clinical trials, and an expanding panel of targeted drugs and treatment options. Access to adequate molecular diagnostics and drugs is crucial to have an impact and move towards implementation in the national healthcare systems. In Norway, a precision cancer medicine ecosystem has been built in recent years [1]. Next-generation sequencing of tumour tissue and circulating tumour DNA (ctDNA), gene expression profiling, and whole genome sequencing are being implemented in cancer diagnostics worldwide, which has resulted in the identification of a number of specific molecular alterations that drive malignancies. This subsequently enables targeted treatment of specific cancer indications. Although targeted drugs are approved for specific tumour

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types, the same molecular alterations can also be present in multiple other tumour types, where the efficacy of the treatment is still not tested, typically due to the rarity of the alterations or a low incidence of the tumour type. While an increasing number of new anti-cancer drugs targeting specific molecular alterations enter the market annually, access to these therapies is still unequal. This particularly affects patients with the poorest prognosis who have exhausted all lines of standard-of-care therapies, those with tumours carrying rare mutations, and patients with rare cancers or carcinoma of unknown primary. This is now investigated in pragmatic national clinical trials such as the DRUP trial (Drug Rediscovery Protocol) in the Netherlands [2] and a family of similar trials in several European countries [3, 4] including the IMPRESS-Norway trial [5].

The primary objective of the IMPRESS-Norway trial is to facilitate patient access to commercially available targeted anti-cancer therapies, and to describe anti-tumour activity and toxicity of targeted therapies. Secondary objectives include further analysis of treatment responses and biomarker analysis. Detailed trial design and trial objectives, including the statistical analysis plan, have been published previously [5]. The trial is still recruiting patients, and the final data analysis will be presented at the later time point. This study reports on the primary endpoint of the IMPRESS-Norway trial per October 1, 2023.

Patients and methods

Study design and endpoints

IMPRESS-Norway is a national, investigator-initiated, prospective, open label, non-randomised, combined basket and umbrella trial. The trial includes patients with incurable progressing cancer disease with no further standard therapy available. Patients are included into treatment cohorts based on tumour type, molecular alteration, and treatment used. The trial uses a Simon two-stage model for adaptive cohort expansion while minimising the number of patients required [6].

Primary study endpoints are: 1) percentage of the patients included in the trial based on their molecular profile, and 2) disease control rate (DCR) defined as objective complete response (CR), partial response (PR) or stable disease (SD) at 16 weeks (W16) after treatment initiation according to established response criteria.

Secondary study endpoints include supportive efficacy analyses, and in this short report, we also include data on DCR in the whole treated population.

All patients who had completed the molecular screening are used for calculating the percentage of patients included and treated in the trial.

The response evaluable population consists of the subset of patients with measurable disease according to established response criteria, and is used to calculate the DCR at W16 after treatment initiation (primary endpoint). Clinical evaluation of unequivocal progressive disease (PD) was accepted as evaluation method in case of inability to perform radiological evaluation. Patients that stopped treatment due to toxicity, withdrawal or death without PD before W16 evaluation, were excluded from the primary endpoint analysis, while patients with progression as defined by established response criteria prior to W16 were included.

For the secondary endpoint analysis, we performed DCR analysis on all included patients who started treatment without major protocol deviations and who received at least one dose of therapy.

All patients had a minimum follow up time of 16 weeks.

Patient population and treatment assignment

Adult patients with advanced incurable malignancies, including haematological malignancies, are eligible for inclusion. All patients must meet study defined inclusion and exclusion criteria and sign an informed consent for molecular screening. Second, drug specific informed consent is obtained prior to treatment initiation, based on molecular screening results and alocated treatment, and after progression on all standard anti-cancer treatment. All patients must meet drug-specific inclusion and exclusion criteria and have clinical or radiological progression as assessed by treating physician before treatment start.

Due to limited capacity of molecular profiling, patients with rare cancer types with few treatment options and patients with tumours having an increased probability of finding actionable alterations, had a screening priority at study initiation. However, screening capacity is continuously increasing, and we expect to screen all referred patients by the end of 2024.

The comprehensive molecular profiling of archival tumour tissue is performed using the Illumina TruSight Oncology 500 (TSO500) gene panel, and screening is reimbursed as part of public healthcare in Norway. In addition, ctDNA analysis by Roche FoundationOne Liquid CDx assay (Foundation Medicine, Inc.) was performed in the first 500 screened patients, as well as for patients with no available tumour tissue and where new biopsies could not be collected. Additional diagnostic tests, such as immunohistochemistry (IHC), fluorescence *in situ* hybridisation (FISH), or other molecular/diagnostic tests, can be used to confirm molecular findings. All screened patients are discussed at the Virtual National Molecular Multidisciplinary Tumor Board. If a targetable molecular alteration is identified and a matching trial drug is available, the patient is offered inclusion in the treatment phase of the study. Currently, 24 different drugs are available in IMPRESS-Norway, of which three are available only for haematological malignancies (Supplementary Table 1).

Efficacy assessments

Patients included in treatment cohorts are evaluated at treatment weeks 8, 16, 26, and every 3 months thereafter. Response is evaluated by RECIST v1.1 [7] in solid tumours, RANO [8] in brain tumours, IWG-ELN, IMWG criteria [9, 10] and CHESON/Lugano recommendations [11] in haematological cancers and non-Hodgkin lymphoma, respectively, and iRECIST [12] is used for

immunotherapy evaluation. Patients are receiving treatment until disease progression, unacceptable toxicity, death, consent withdrawal or withdrawal by the decision of the study investigator.

Data collection and statistical analysis

Data are collected from electronic report case form (eCRF) Viedoc. R version 4.3.2 was used for statistical analysis. Patient characteristics and tumour responses were summarised using descriptive statistics.

Results

Patients

IMPRESS-Norway opened for accrual at April 1, 2021. By October 1, 2023, 1,167 patients had completed molecular profiling and subsequent evaluation for inclusion in the study treatment phase. The median age for patients included into molecular screening was 58 years (range 18–84 years), the majority of the patients had Eastern Cooperative Oncology Group (ECOG) Performance Status 0–1, and equal number of female and male patients were included. Patients included in the treatment phase had a median age of 60 years (range 19–80 years), 81% had ECOG 0–1, and 55% were female. The most common cancer types included were colorectal cancer, lung cancer, and cholangiocarcinoma. Baseline patient characteristics of screened patients and patients included in the treatment phase are shown in Supplementary Table 2.

A total of 358 of all screened patients (31%) had an actionable molecular alteration and a matching targeted drug eligible for inclusion in the treatment phase of the study. Of these, 138 patients were still receiving standard treatment and are candidates for inclusion upon progression on standard therapy. Thirty-four patients did not meet the criteria for initiating treatment, commonly due to disease progression and general deterioration of their condition during screening, or they did not meet drug-specific inclusion criteria. By October 2023, 186 patients started treatment, 16 patients had follow up time less than 16 weeks, and 25 patients stopped treatment without detected progression before W16 due to toxicity (*n* = 18), death

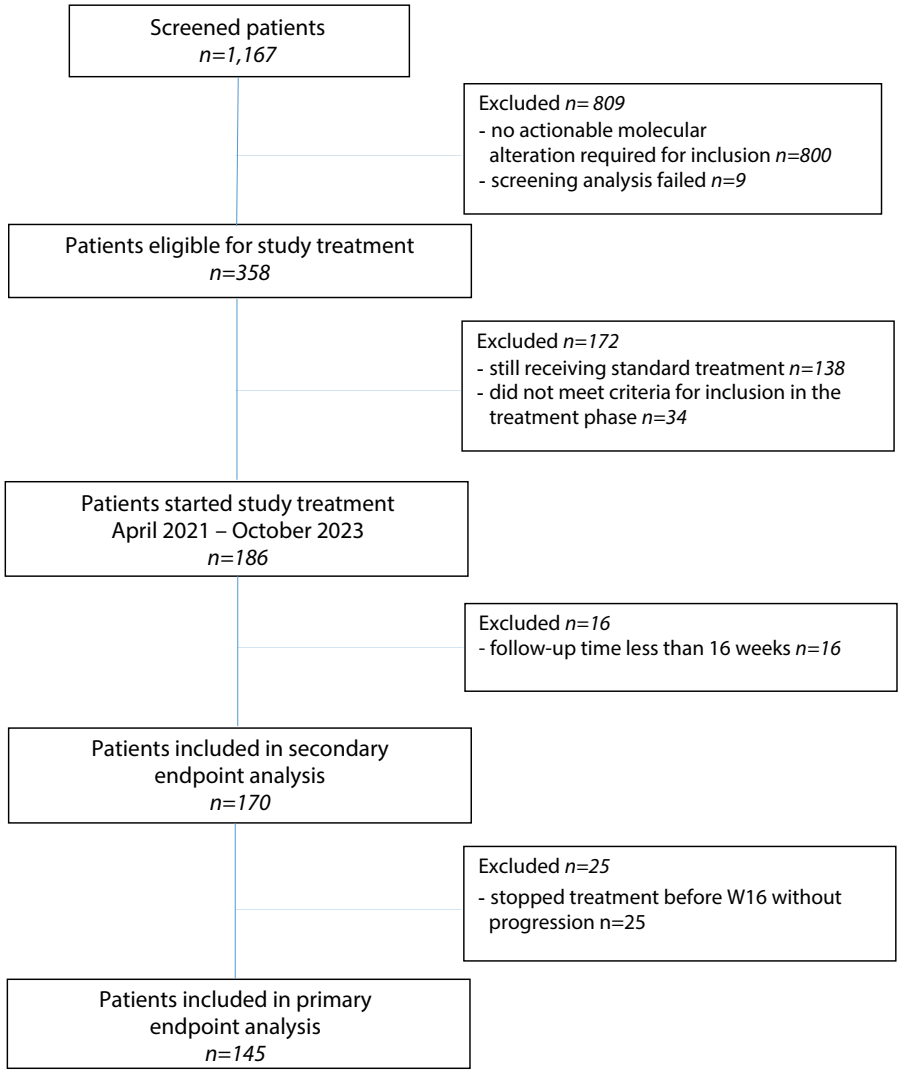


Figure 1. Flow diagram of patients included in the IMPRESS-Norway analysis.

Table 1. Number of patients treated with different treatments/treatment combinations.

Study treatment/ treatment combination	Number of patients treated, *n* = 170
Trametinib	35
Pertuzumab and trastuzumab	31
Atezolizumab	25
Cobimetinib and vemurafenib	19
Alpelisib	18
Trametinib and dabrafenib	14
Atezolizumab and bevacizumab	7
Pemigatinib	7
Vismodegib	5
Olaparib	2
Imatinib	2
Alectinib	2
Alpelisib and fulvestrant	1
Entrectinib	1
Capmatinib	1

(*n* = 2) or withdrawal (*n* = 5). Thus, response evaluable population consists of 145 patients. A schematic overview of screened patients and patients included in the treatment phase and efficacy analysis is shown in Figure 1. Patients have been included in 109 different treatment cohorts based on tumour type, genomic alteration, and targeted therapy. A complete list of used therapies and the number of patients treated is available in Table 1.

Disease control rate at Week 16, preliminary results

The primary endpoint, DCR at W16 was 40% (58/145); 1 patient (<1%) had CR, 17 (12%) patients PR, and 40 patients (28%) had SD. Eighty-seven patients (60%) had PD at W16. Progression was radiologically confirmed in 50 patients, while 37 patients had unequivocal clinical PD.

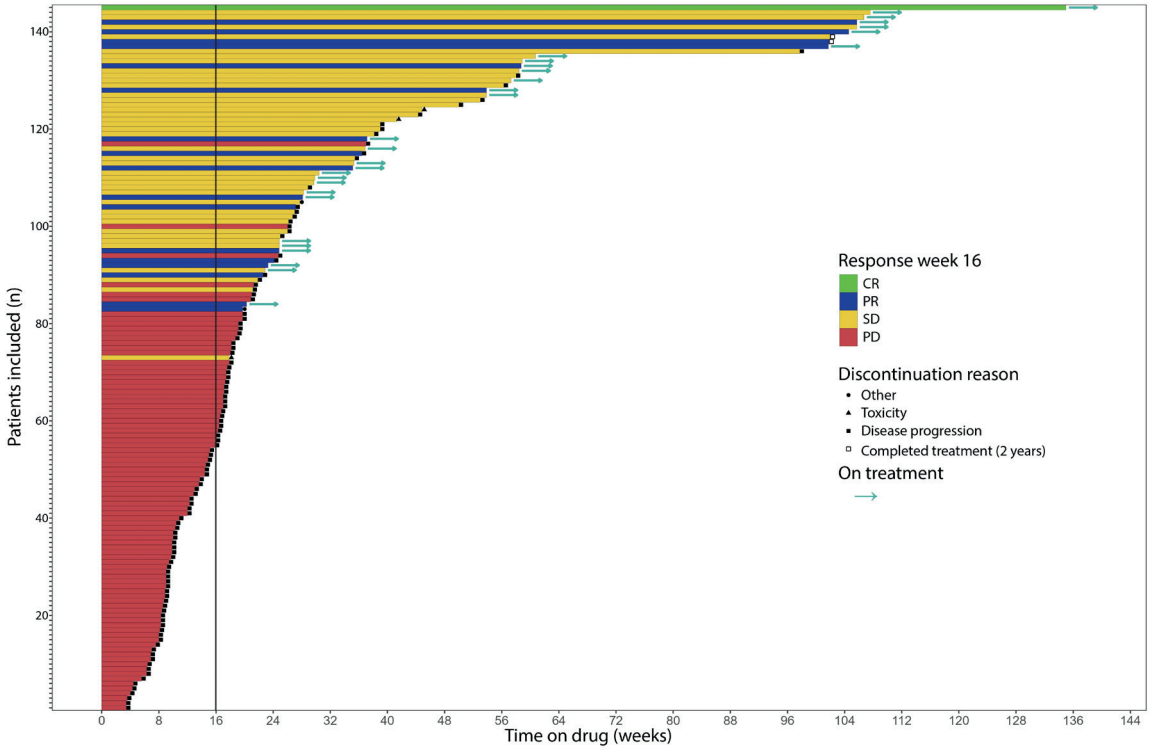
The secondary endpoint, DCR at W16 in all treated patients was observed in 34% (58/170) of the patients.

Preliminary results are summarised in Figure 2.

Discussion

Preliminary results from the IMPRESS-Norway trial show that comprehensive molecular tumour profiling is feasible and confirm the presence of targetable molecular alterations, leading to additional experimental targeted treatment in around 30–40%

a)



b)

Disease control rate at W16, n=145	
CR+PR+SD	58/145 (40%)
CR	1 (<1%)
PR	17 (12%)
SD	40 (28%)
PD	87 (60%)

Figure 2. Preliminary results. a) Swimmer plot of the time on treatment, observed response at W16, and reason for treatment stop in response evaluable population, n=145. b) Disease control rate among response evaluable patients at W16, n=145

of all screened patients, either as a treatment in IMPRESS-Norway, other studies or early access programmes. This is in accordance with earlier published meta-analysis and other similar studies, where the percentage of patients having targetable alterations varied from 30 to 88% [13–15].

The observed DCR at 16 weeks of treatment for evaluable patients, was 40%. The first European precision medicine trial, SHIVA, reported no clinical benefit in 99 treated patients in 2015 [16], the MOSCATO 01 trial reported objective response rates of 11% [17]. More recently, the DRUP trial reported clinical benefit rate at 16 weeks of 34% [2], whereas the CoPPO trial in Denmark and first results from the MyPathway trial in the United States reported objective response rates in 15 and 23% of treated patients, respectively [18, 19].

IMPRESS-Norway, like other precision medicine trials, has limitations that need to be taken into consideration when interpreting results. The majority of the trials are non-randomised lacking a control group. There are several differences between the precision medicine trials, for example, molecular profiling tests used for inclusion, changes in understanding and interpretation of molecular findings, and access to targeted treatments over time. Study endpoints varied from progression free survival, response rates, clinical benefit and DCR. Therefore, comparison and the interpretation of results may be difficult. However, our results seem to be in line with results reported in later trials.

Due to limited availability of molecular profiling and access to drugs at IMPRESS-Norway initiation in 2021, patients with an increased probability of finding targetable alterations were prioritised for screening, indicating a certain degree of patient pre-selection to the trial. This could lead to a higher percentage of actionable findings than if an unselected population was screened. In some of the other studies, like the DRUP trial, all patients were pre-selected prior to referral for inclusion. On the other hand, in IMPRESS-Norway, targetable alterations were defined by the availability of matching drug, meaning potentially targetable alterations that were not acted upon, were not counted as actionable, indicating that our reported percentage could be higher. As the larger gene-panels are becoming more available and a number of targeted therapies is increasing, it is expected that a higher proportion of patients will have actionable targets within the study.

In conclusion, the introduction of national comprehensive molecular diagnostics has ensured additional treatment options for approximately one-third of patients screened in IMPRESS-Norway trial. Increased knowledge on molecular targets, access to comprehensive molecular diagnostics, and targeted treatments contributed to the observed increased benefit compared to the first reported precision medicine trials.

IMPRESS-Norway continues to recruit patients and collaborates with other DRUP-like clinical trials across Europe, such as the ProTarget trial in Denmark [3] and FINPROVE in Finland [4]. Through the European initiatives PCM4EU [20] and PRIME-ROSE [21], DRUP-like clinical trials have built a distributed clinical trial network that addresses national priorities while collaborating internationally for scale and impact.

Authors contribution

GLF, ESB, ÅF, J-ÅL, RH, SS, HEGR, KT, ÅH, BTG have provided study concept and design. KP, GLF and ÅH have provided manuscript draft. KP, GLF, ÅH and RSF have analysed and interpreted data. All authors have contributed with data acquisition and critical revision of the manuscript.

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Disclosure statement

Katarina Puco reports receipt of honoraria for advisory board participation from Astellas, MSD, Pfizer and Bayer; honoraria for speaker services from Astella, Ipsen, Janssen-Cilaq, AstraZeneca and Merck.

Sigmund Brabrand reports receipt of honoraria for speaker services from Astellas and Pfizer.

Eli Sihn Samdal Steinskog reports receipt of honoraria for speaker services from Pfizer, Bayer and Roche.

Line Bjørge reports receipt of honoraria from AstaZeneca, GSK and MSD and institutional funding from AstraZeneca.

Sebastian Meltzer reports receipt of honoraria advisory board participation from GSK.

Randi Hovland reports receipt of honoraria advisory board participation from AstraZeneca and Novartis; honoraria for speaker services from Sanofi and Janssen-Cilaq.

Kjetil Taskén reports receipt of honoraria advisory board participation from Exscientia and Serca Pharmaceuticals and stock and other ownership in Serca Pharmaceuticals and Leididi.

Gro Live Fagereng, Pitt Niehusmann, Egil Støre Blix, Åse Haug, Cecilie Fredvik Torkildsen, Irja Aida Oppedal, Åsmund Flobak, Kajsa Anna Margareta Johansson, Geir Olav Hjortland, Astrid Dalhaug, Jo-Åsmund Lund, Bjørnar Gilje, Marte Grønlie Cameron, Ragnhild S. Falk, Sigbjørn Smeland, Hege Elisabeth Giercksky Russnes and Åslaug Helland did not report any possible conflicts

of interest.

Merck (CrossRef Funder ID: 10.13039/100009945) reviewed this manuscript for medical accuracy only before journal submission. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

Data availability statement

The full clinical dataset consists of de-identified patient-level data obtained from VieDoc. The sponsor and data owner is Oslo University Hospital. Access to full raw patient-level data is limited, but project partners can apply for access through the data and biobank committee of the trial, in accordance with Data Privacy and Ethical Approval for the study project. All authors have full access to complete study data, study analysis performed, tables and figures. The study protocol, including a statistical analysis plan, is available.

Ethics declaration and trial registration form

This study is conducted in accordance with Good Clinical Practice and other relevant research practices, legal requirements and ethical guidelines. The study is approved by the Regional Committees for Medical Research Ethics South East Norway (#200764) and registered at ClinicalTrials.gov (NCT04817956). Patient informed consent was obtained for each patient prior to study inclusion.

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LETTER

A national precision cancer medicine implementation initiative for Finland

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To the Editor – The adaptation of nationwide genomic profiling and personalized cancer therapy in Finland has been challenging due to the lack of a uniform framework and funding. Meanwhile, increasing evidence demonstrates a clear benefit from precision medicine in cancer care [1, 2] while the need for more efficient therapies in hard-to-treat cancers is evident. Equally a challenge for these cancers remains in the lack of randomized trials.

Work to implement precision cancer medicine at a national level in Finland, began in 2021 led by Helsinki University Hospital (HUS) and FICAN South to meet three goals: (1) to implement genomic profiling and precision cancer medicine as standard of care, (2) establish equal access to molecular diagnostics and clinical trials in precision medicine, (3) to increase the number of precision cancer medicine trials and open a national DRUP (Drug Rediscovery Protocol)-like clinical trial.

A multidisciplinary **study team** including oncologists, hematologists, gynecological oncologists, pathologists, cancer researchers, molecular and clinical geneticists was formed. This nationwide working group had the common aim to enable access to personalized cancer therapy for all patients in Finland irrespective of their residence. Early discussion amongst this initiative urged the need to equally engage stakeholders for reimbursement and sustained funding. Of equal importance was to explore the possibility of a public-private partnership for drug access and possibilities for shared pay for benefit funding. Political and financial support have revealed to be the most challenging steps within this initiative as consistent public funding is still lacking. Without international coordination with other major precision cancer medicine initiatives, especially the Dutch DRUP trial, the implementation would have been impossible.

Tertiary care for cancer treatment in Finland is centralized to five university hospitals each governing a capture area of 0.7 to 1.7 M inhabitants. Each University hospital has a **regional cancer center** (FICAN South, West, East, North and Mid) that aims to promote equal access to diagnostics and treatment and, thus, the FICANs have played a major role in the precision cancer medicine initiative. During the past 3 years, we have therefore built and raised funding for an ecosystem that includes four major working groups: molecular diagnostics, data storage and secondary use, reimbursement, and finally the national DRUP like trial. National infrastructure for precision cancer diagnostics requires all five university hospitals across Finland, and these in turn facilitate the use of advanced molecular diagnostics, operate local Molecular Tumor Boards (MTBs) and participate in our national MTB. The national MTB operates digitally (Teams) on a weekly basis evaluating cases that have been pre-screened for FINPROVE by the local MTBs (Figure 1).

To facilitate precision cancer medicine implementation and patients' access to targeted anti-cancer drugs, FINPROVE was launched in August 2021. FINPROVE is a national investigator-initiated interventional phase 2 trial that opened for inclusion in January of 2022 first in Helsinki University Hospital, and now the trial is open in all five University Hospitals. Smaller regional hospitals operate through their governing university hospital to include patients in FINPROVE. The trial is coordinated with and modeled on, the Dutch DRUP trial, which has been successful in the uptake of precision medicine nationwide, due to high inclusion rates resulting in significant patient benefit and exploring new national reimbursement models [3, 4]. FINPROVE is also aligned with similar trials in Denmark (ProTarget), Norway (IMPRESS) and Sweden (FOCUSE). Importantly, EU has funded two large precision cancer initiatives facilitating collaboration within countries working on these trials and precision medicine: PCM4EU and PRIME-ROSE. Both initiatives comprise of projects for research on control cohorts, use of real-world evidence,

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reimbursement models and health economics, ethics, legal aspects, and data governance. Partly due to this, our national initiative has gained substantial interest in patient organizations, private companies and stakeholders involved in reimbursement strategies for new therapy/drug indications. The majority of funding is covered through the University Hospitals but of equal importance have been private trusts. Additional funding comes from company contributions for drugs, and costs per-patient in FINPROVE.

The FINPROVE ecosystem is currently scaling to include regional hospitals as sites in aiming at nationwide equality for both molecular profiling and inclusion to precision medicine trials. The major challenge for larger uptake of genomic profiling is the lack of public reimbursement for advanced molecular panels and the diversity of genomic analysis used. For this reason, access to expensive panels has not been uniform and requires ongoing discussions with public stakeholders as agnostic indications and approved targeted agents have entered standard-of-care with increasing pace. Actionable targets are confirmed centrally upon trial entry. Yet for screening all University Hospitals use commercial panels (e.g. TruSight Oncology 500, Foundation One Cdx, Oncomine Comprehensive Assay Plus) while validating in-house panels that will hopefully be available for all cancer patients in Finland in the future as national guidance for screening still requires implementation.

By November 2023, the national MTB has evaluated 450 patients with molecular profiling results for inclusion in the FINPROVE trial and other ongoing studies. Of these, more than 100 (25%) patients have been offered treatment within FINPROVE which consists of cohorts defined by a specific molecular profile (eg. mutation, fusion, or amplification) and drug. The trial is a cluster of cohorts that follows a Simon two-stage model with Stage 1 consisting of a cohort of eight patients in a combined umbrella and basket design. Positive cohorts (≥5 of 24 responsive patients, Stage 2) may expand into larger cohorts (Stage 3) from which discussions on drug reimbursement can be based on. Currently reimbursement is only case-based and models for extension of indications are to be explored. When the trial started, eight drugs were offered from Roche, but continuing discussions with other companies have resulted in the inclusion of drugs from Bayer, Novartis, Eli Lilly, and Janssen, adding up to 13 drugs at the end of 2023. By the end of the second quarter in 2024, we foresee to have additional companies, and 18 drugs in the trial, improving patient inclusion and, hopefully, benefits for the patients.

Through this initiative, we now have an ecosystem that has a mutual aim to increase clinical trial access for cancer patients across Finland, harmonize molecular testing, and equalize standard-of-care. Patients with progressive cancer disease can now be referred to their governing University Hospital irrespective of where they live and have the possibility for advanced molecular cancer diagnostics [5]. Through the national MTB, an increasing number of cancer patients now have access to more treatment lines, both for standard-of-care and experimental drugs, beyond previous availability. The whole ecosystem strengthens translational research and innovation through extensive data generation, biobanking and national registries. All University Hospitals have the competence to utilize their data lakes to promote drug efficacy data, data on health economics for future technology assessments, new diagnostic methods and re-enforce international competence through AI.

Future plans for this precision cancer medicine ecosystem include expanding the drugs included in FINPROVE while also allowing drug combinations especially for hematologic malignancies. Moreover, a deeper understanding on the molecular underpinnings of response and resistance of tumors to targeted treatments is urgently needed to enhance precision oncology approaches as it is unknown why some of the patients with the same molecular alteration respond and some fail to respond to the same therapy [6].

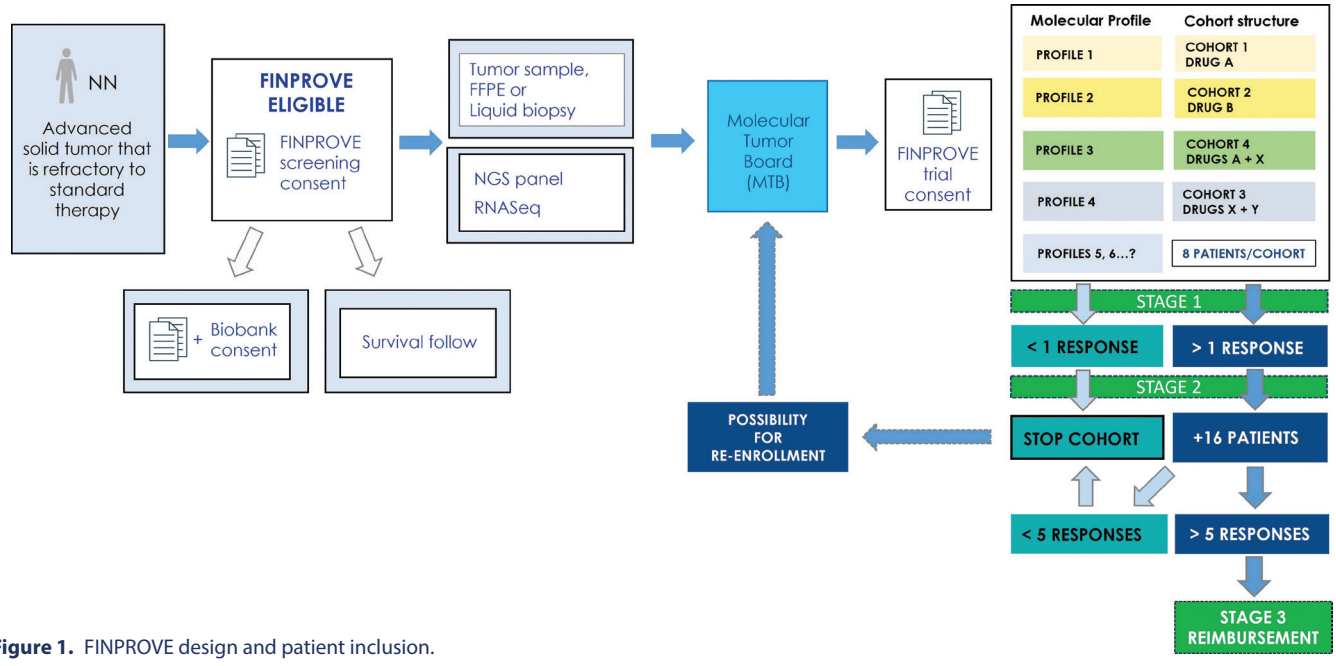

Figure 1. FINPROVE design and patient inclusion.

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Author contributions

KJ, EA, SI, OSK and MT from all five university hospitals share equal contribution. Important discussions and contributions to the initiatives were made by all authors. Authors have approved the final version of the text.

Competing interests

Participation in the FINPROVE Public–Private Partnership is regulated by a consortium agreement that handles conflicts of interest and regulates interaction with the publicly funded infrastructure FINPROVE consortium (co-ordinating PI KJ) has company contributions from Roche, Novartis, Bayer, Janssen and Eli Lilly. The coordinating author (KJ) reports the following outside of this work, advisory fees: Ipsen, BMS, Roche, MSD, Bayer, Novartis and stock ownership Faron Pharmaceuticals.

The trial is registered both in EudraCT 2021-000689-14 and ClinicalTrials.gov NTC05159245 registries.

Ethics approval

The protocol has been approved by the institutional review boards and independent ethics committees of the participating centers. The study is performed in accordance with the Declaration of Helsinki and is conducted in compliance with the International Conference on Harmonisation on Good Clinical Practice.

Data availability statement

The trial is on progress.

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SHORT REPORT

The French multicentric molecular analysis platforms and personalized medicine trials MOST, MOST Plus and MEGAMOST

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ABSTRACT

Background and purpose: In this manuscript we describe the academic French multicentric molecular analysis platforms including PROFILER, promoted by Centre Léon Berard, and the multicentric personalized medicine trials MOST, MOST Plus and MEGAMOST.

Patients/material and methods: MOST, MOST Plus and MEGAMOST comprise 14 cohorts with different targeted agents and immunotherapies.

Results and interpretation: PROFILER has recruited 5,991 patients in 10 years, MOST and MOST Plus 875 patients since 2014 and MEGAMOST 172 patients since 2020, and are still ongoing. We provide a description of the local, national and international implications of these initiatives, and we review the results of the sorafenib and olaparib cohorts.

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Personalized oncology; molecular analysis; oncology; sorafenib; olaparib

Introduction

The first generation of personalized medicine umbrella or basket trials in France allowed 10–20% of patients to receive a targeted treatment based on molecular analysis (mainly targeted sequencing and comparative genomic hybridization (CGH)) [1–3]. It is estimated that 40–50% of patients would benefit from theoretical orientation if more treatments were available and accessible [4, 5]. Drug Rediscovery Protocol (DRUP)-like trials have the potential to increase the number of compounds and molecular markers to orient patients to targeted treatments. Centre Léon Bérard (CLB) and partnering sites have developed an environment combining multiple types of molecular analysis and orientation to academic DRUP-like trials called MOST and MEGAMOST for patients with advanced or metastatic cancers.

Molecular analysis and sequencing programs

Several molecular analysis programs are currently running in our hospital resulting in multiple levels of molecular information (Table 1). Most of the platforms are multicentric, either regional in Rhône Alpes and centralised at CLB as for ProfiLER01, or national and centralised in dedicated sites (including CLB), as for FMG2025.

ProfiLER screening programs

The ProfiLER01 (NCT01774409) is a multicentric, prospective and non-randomised ongoing program. ProfiLER is dedicated to adult patients with advanced/metastatic cancer who progressed after at least one line of standard treatment. The current molecular analysis includes the identification of single nucleotide variants (which evolved across three different panels over time), copy number alterations (using CGH array), tumour mutational burden, microsatellite status (both implemented since 2023) and oncogenic fusion using in-house genomic workflows. ProfilER02 (NCT03163732) included FoundationOne® CDX panel of 324 genes (under review). This is a multidisciplinary effort including the molecular biology platform, the Gilles Thomas Bioinformatics Platform, the biosamples management platform, the clinical staff and the molecular tumour board. The molecular tumour board is made up of medical oncologist, pathologist, molecular biologists, bioinformaticians and data scientists meeting every week to recommend matched molecular-targeted agents including immunotherapies and including those accessible in clinical trials [6].

The ProfiLER01 program enrolled 5,991 patients between February 2013 and November 2023 and were ongoing at this time. On the basis of these data, our team has previously described the molecular characteristics of several population

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Table 1. Molecular screening programs at Centre Léon Bérard.

Program	Type of data	Organisation	Tools used for interpretation
ProfiLER	Tumour target DNAseq, RNAseq, CGH	Rhône Alpes: Data, analysis & MTB centralized at CLB	In house + Open source
FMG2025	WGS, RNAseq	France, reports at CLB for MTB	National
PRISMportal	ctDNAseq	France. In Rhône Alpes: Data, analysis & MTB centralized at CLB	Foundation medicine
PLANET	ctDNAseq, tumor WES, RNAseq	CLB, sequential analysis	In house

ctDNAseq: circulating DNA sequencing; WES: Whole Exome Sequencing; DNAseq: DNA Seqencing; RNAseq: RNA Sequencing; CGH: Comparative Genomic Hybridisation; MTB: molecular tumour board; CLB: Centre Leon Berard; CGI: Cancer Genome Interpreter.

including patients with gastro-oesophageal cancers [7], patients with alterations in homologous recombination-related genes and distinct platinum response in metastatic triple-negative breast cancers [8], patients with primary brain tumours [9], refractory gynaecological cancers [10], metastatic sarcomas [11] and paediatric tumours [12].

Other molecular analysis programs

The 'France Génomique plan 2025' (FMG2025) provides whole genome sequencing (WES) and RNAseq for patients with refractory diseases. Analyses are performed on two platforms: Auvergne Rhônes Alpes Genomique (AURAGEN) in Lyon covering the analysis of Southern France and Sequencing Omics Information Analysis (SeqOIA) in Paris covering the analysis of Northern France [13]. It proposes extensive molecular testing with WES and RNA sequencing for multiple diseases including 60 types of rare diseases, uncharacterized suspected genetic predispositions and eight indications in oncology: refractory cancers, rare cancers, cancer of unknown primary and haematology. The first patients were included in October 2019 and up to early 2023, 8,447 reports were generated including 1,969 patients with cancer.

Another program, PRISM-Portal, evaluates the impact of ctDNA at the start of metastatic disease, during treatment and/or at progression. The proportion of patients with ctDNA sequencing has helped guide therapy.

The PLANET program (NCT05099068) aims to generate sequential molecular analysis for patients treated with standard therapies, including detection of mutations, amplifications, insertions/deletions, microsatellite instability, mutational burden and expression alteration using RNA Sequencing either on tumor and/or liquid biopsies.

Genomic-driven clinical trials

MOST-MOST Plus and MEGAMOST

MOST-MOST Plus and MegaMOST trials are composed of multiple treatment cohorts defined by the combination of a targeted treatment and a biomarker derived from molecular profiling. New cohorts are opened on a regular basis through the integration of new study treatments, generally in indications unexplored by pivotal pharma-initiated trials. Both have adaptive Bayesian approach, futility interim analysis and a target of 50 patients analysed for the primary endpoint for each cohort [14]. A Bayesian approach allows updating knowledge gradually rather than restricting revisions in a trial design with fixed sample sizes. The main selection criteria include adult patients with metastatic or unresectable solid tumours of any type, not amenable to curative treatment, and those who previously received at least one prior systemic treatment regimen.

MOST

The MOST program (NCT02029001) started in 2014 with a multi-arm, genomic-driven Phase II trial, conducted using a randomised discontinuation design. This is a way to evaluate the efficacy of molecular targeted agent oriented towards a matched molecular alteration in a randomized fashion. After an induction period of treatment of 12 weeks, patients with stable disease are randomly assigned (1:1) to continuation or interruption of matched therapy defining the maintenance period (Figure 1). Between 2014 and November 2023, we enrolled 427 patients in five cohorts with the molecular targeted agents lapatinib, sorafenib, everolimus, pazopanib, or nilotinib oriented by predefined somatic alterations (Table 2). The trial is running in six French sites (Centre Léon Bérard, Hospices Civil de Lyon, Institut Curie, Institut Paoli Calmettes, Oncopole Toulouse, Institut Bergonié). The primary endpoint is progression-free rate at 16 weeks after randomisation.

The MOST sorafenib cohort was composed of 151 patients with at least one of the following molecular alterations: mutations or amplification/translocation in VEGFR1-3, PDGFRB, FLT3, BRAF (excluding V600E), CRAF, HRAS, KRAS, or RET, and/or cognate ligands. For the induction period, 35 patients had SD at 12 weeks. The progression-free rate at 16 weeks after randomisation was 65% [95%CI 43.4–83.7] in the continuation arm, with a significant increase in PFS (5.6 months [95%CI 1.97–6.77] versus 2.0 months [95%CI 1.61–3.91], *p*-value = 0.0231). The progression-free rate in the interruption arm was 25% [7.8–48.1]. The median survival was also improved from 4.3 [95%CI 8.9-23.8] in the interruption arm to 8.0 months [95%CI 3.5-15.2] in the continuation arm, *p*-value = 0.0857. It suggests that sorafenib matched to molecular alterations improved the outcome of patients with SD compared with its interruption. Grade 3 or higher sorafenib-related adverse events were reported in 67 patients (46.2%), as hypertension, vomiting, fatigue, hand and foot syndrome [15].

The nilotinib cohort continues only for advanced pigmented villonodular synovitis (TGCT/PVNS), a group of locally aggressive tumours with activation of the CSF1R pathway [16]. The everolimus, pazopanib and lapatinib cohorts are closed to enrolment and under analysis. Although there is a randomisation for comparative analysis, a potential limitation in the

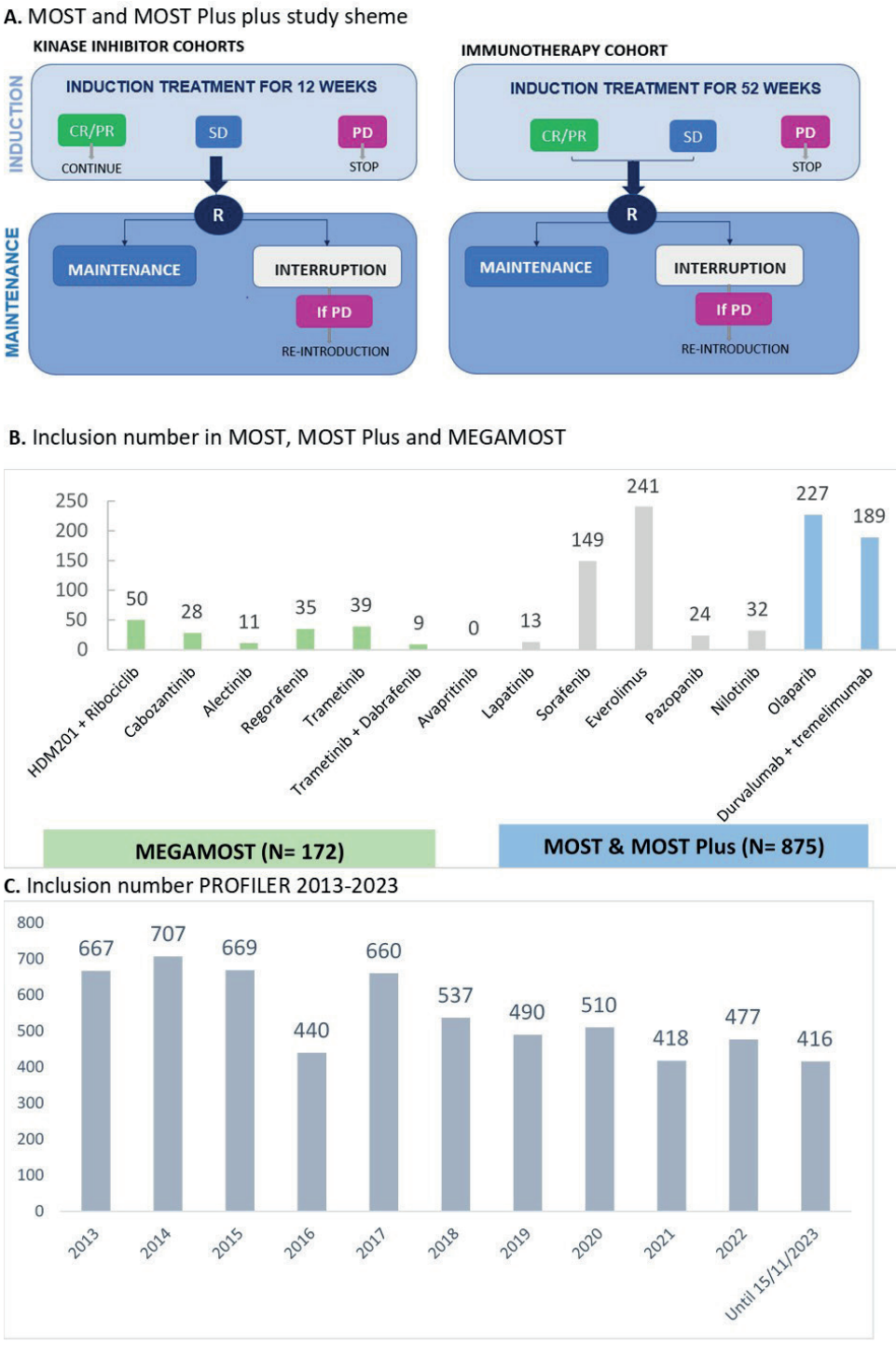

Figure 1. A. the MOST plus study scheme and B. Inclusions in MOST, MOST Plus and MEGAMOST In blue (MOST and MOST-Plus) or green (MegaMOST): ongoing cohorts, in grey: cohorts closed. Of note, avapritinib cohort was opened in October 2023. C. Inclusion number PROFILER 2013–2023. MTT: Molecular Target Therapy; CR: complete response; PR: partial response; SD: stable disease; PD: progression disease; R: randomisation.

interpretation of the results of the MOST trial is that it does not include a control group of patients not driven on prespecified genomic alterations.

MOST Plus

MOST Plus is an amended version of MOST (NCT02029001) with the addition of 2 cohorts of patients treated with olaparib or the combination of durvalumab and tremelimumab (Table 2). The induction period of treatment is 12 weeks for olaparib and 52 weeks for immunotherapy before randomisation of patients with stable disease (for olaparib) or stable disease and objective response (for D+T cohort). The MOST Plus durvalumab and tremelimumab is ongoing and recruited 189 patients up to November 2023. The MOST Plus olaparib cohort, presented at ESMO2023, included 213 patients with somatic or germline

Table 2. Cohorts of the MOST+ and MEGAMOST trial.

Molecular alterations*	Study drug's name and Dosage regimen	Eligible histological tumour types	Partner
Documented amplification of • CDK6 and/or CDK4 • and/or CDKN2A homozygous deletion • and/or amplification of CCND1 • and/or CCND3 with no deletion/losses more than single copy of RB1 by copy number and P53 wild-type	**ribociclib** 200 mg/day, QD, 2 weeks on/1 week off, PO + **HDM201** 120 mg, Q3W, PO	Adult any solid tumours (excluding gliomas)	Novartis
AXL, MET, VEGFR, VEGF, RET, ROS1, MER, TRKB, TIE-2 • and/or Tyro3 activating mutation • and/or amplification • and/or NTRK translocation	**cabozantinib**, 60 mg/day, continuous, PO	Adult with any solid tumour	Ipsen
Activating ALK alterations: • translocation • or selected mutations (for instance R1275Q, F1245C, F1174X or listed Appendix 9)	**alectinib**, 600 mg BID, PO	Adult patients with advanced or metastatic • Cohort 1: Inflammatory myofibroblastic tumors • Cohort 2: Neuroblastoma, • Cohort 3: other tumours with ALK alterations.	Roche
Activating mutation and/or amplification of VEGFR1-3, TIE-2, KIT, RET, RAF1, BRAF (other than V600 mutations), CRAF, HRAS, KRAS, PDGFR, FGFR1-2, FLT3 • and/or CSF1R • and/or amplification of the ligands • and/or biallelic inactivation of SMAD4	**regorafenib** 160 mg, once daily, 3 weeks on/1 week off, PO	Adult with any solid tumours	Bayer
Activating mutation and/or amplification of KRAS (except KRAS G12C), NRAS, HRAS and/or MAP2K • and/or biallelic inactivation [§] of NF1 • and/or activating mutation PTPN11 • and/or amplification or translocation of BRAF	**trametinib** 2 mg/day, continuous, PO	Adult with solid tumour, excluding melanoma Lung cancers with KRAS G12C mutation, CRC and PDAC with KRAS mutations.	Novartis
BRAF V600 mutation	**trametinib** 2 mg/day, continuous, PO + **dabrafenib** 150 mg BID, PO	Adult with solid tumor, excluding Melanoma, Lung cancers, CRC.	Novartis
Activating mutations of KIT exon 17 • or PDGFRA exon 18 associated • or not to mutation on KIT exon 11 • or PDGFRA exon 12/14	**avapritinib** 300 mg/day, continuous, PO	Adult with any solid tumour	Blue Print
Mutations of ABL1, KIT, PDGFRA, PDGFRB, DDR1, DDR2, CSF1R, or amplification/translocation of the genes and/or ligands	**nilotinib** 400 mg/day continuous, PO	Only pigmented villonodular synovitis, not amenable to curative treatment	Novartis
Mutations or amplification of the • PIK3CA, PIK3R1, AKT1, AKT2, mTOR, RICTOR, RAPTOR genes • or with TSC1, TSC2 • or PTEN loss (defined as a complete loss of both gene copies OR loss of one copy + mutation on the other copy OR loss of one copy + loss of expression using IHC)	**everolimus** 10 mg mg/day continuous, PO	Adult with any solid tumour	Novartis
Mutations of VEGFR1-3, PDGFRA, PDGFRB or KIT or amplification/translocation of the genes and/or of the ligands	**pazopanib** 800 mg/day continuous, PO	Adult with any solid tumour	Novartis
Mutations of VEGFR1-3, PDGFRB, FLT3, BRAF (other than V600 mutations), CRAF, HRAS, KRAS or RET or amplification/ translocation of the genes and/or of the ligands	**sorafenib** 400 mg BID continuous, PO	Adult with any solid tumour	Bayer
Mutations or amplifications of HER2	**lapatinib** 1,500 mg/day continuous, PO	Adult with any solid tumour	Novartis
• Mutation only if double hit documented: ATM, BAP1 et BRIP1 • Mutation : BRCA2, BRCA1, RAD51C, PALB2, RAD51D • Loss: BRCA1, BRCA2, ATM and BAP1 • Mutation + heterozygote deletion: BRCA1, BRCA2, ATM and BAP 1	**olaparib** 300 mg BID	Adult with any solid tumour except in prostate, or stomach cancers and except for patients eligible to olaparib's available labels and reimbursements in France	Astra Zeneca
Tumour mutation burden >10 Muts/Mb on liquid biopsy • MSI-High • PD1/PDL1/CTLA4 amplification • MLH1/MSH2/MSH6/PMS2 • POLD1 & POLE mutation or LOH except lung or urothelial or head and neck tumours	**durvalumab** 1,500 mg/day, Q4W, PO + **tremelimumab** 75 mg/day, Q4W, PO	Adult with any solid tumour	Astra Zeneca

mutations in homologous recombination genes such as BRCA1/2, RAD51, PALB2, ATM, etc. (beyond current label in oncology). Among the 213 patients who received olaparib (300 mg, BID), 6% ($n = 14$) had partial response at 12 weeks and 16% had stable disease, with a 3-month PFS rate of 23% (48/213). For patients with partial responses, 8 had breast cancer, 3 pancreatic cancers, and the 3 remaining had prostate, uterine or bladder cancers, most of them harbouring biallelic alterations in homologous recombination genes. Among all patients, 23.6% with PALB2 mutations had partial responses. Grade 3 or higher adverse events were reported in 81 patients (38%) and 14.1% of patients discontinued treatment due to adverse events [17]. Based on this analysis, we recently updated the molecular selection criteria for future patients enrolled in this cohort: alterations on the genes BRCA2, BRCA1, RAD51C, RAD51D, PALB2, BAP1, ATM and BRIP1.

MEGAMOST

MEGAMOST (NCT04116541) is an ongoing phase II, genomic-driven adaptive Master protocol. Patients are assigned to a treatment cohort based on molecular alterations/characteristics detected on tumour samples (from primary tumour or metastatic lesion) or liquid biopsy. MEGAMOST is currently running in six French sites (Centre Léon Bérard, Centre Antoine Lacassagne, Institut Bergonié, Institut Paoli Calmettes, Oncopole Toulouse and Gustave Roussy Cancer Center). Up to November 2023, 172 patients were enrolled in seven cohorts of molecular targeted agents (HDM201 and ribociclib, alectinib, regorafenib, trametinib, trametinib and dabrafenib, avapritinib) in advanced/metastatic solid tumours. The primary objective is to evaluate the activity of selected study drugs for each cohort based on molecular alterations characteristics of the patient's tumour (progression free rate after 3 months of treatment). A Bayesian statistical approach is regularly analysing the efficacy of each cohort. The patients' recruitment is ongoing in each cohort and no publication is already available.

Conclusion and perspectives

The high failure rate of clinical development in oncology is mainly due to the erroneous hypothesis that all patients affected by a similar tumour type would be biologically identical (this is represented by selection criteria of clinical trials oriented on tumour types). The MOST trials are clearly aiming at repositioning molecular targeted agents with a

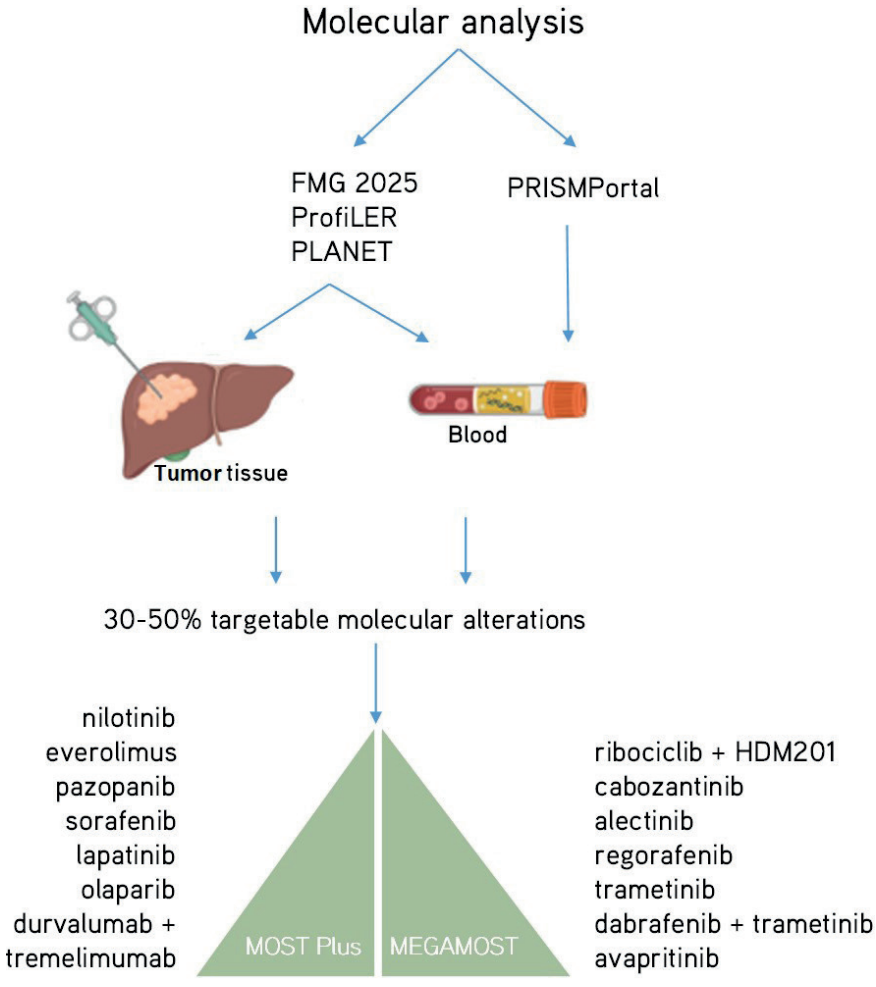

Figure 2. The molecular diagnostic programs are used to orient patients to the MOST Plus and MEGAMOST clinical trials.

personalized medicine strategy (Figure 2). The success of repurposing molecular targeted agents in oncology is supported by the recent analysis of the main factors leading to the best Likelihood of FDA Approval (LoA) for pharmaceutical compounds together with their companion diagnostic tools, namely (1) rare disease therapy (LoA = 17%), (2) development of a treatment with biomarkers (i.e. companion diagnostic tools, LoA = 16%), and (3) prior approval (i.e. repositioning, LoA +3.6%) [18, 19]. When a cohort meets the efficacy endpoint in a cohort of a DRUP-like trial, it can support drug approval and reimbursement in the participating country. For example, nivolumab, an immune-checkpoint inhibitor targeting anti-PD1, obtained approval and reimbursement in the Netherlands on July 1st, 2022, based on a cohort of the DRUP trial evaluating the treatment of dMMR/MSI solid tumours of any origin [5, 20, 21]. Nevertheless, two teams in the PCM4EU consortium showed that up to 40–50% of patients with rare cancers could have a genomic-driven orientation if treatments were available and accessible in the country of the patients [4, 5]. To this end, DRUP-like trials such as MOST trials include a process of public, open, and shared evaluation of the treatment efficacy. The collaboration of several DRUP-like trials on data sharing will support an efficient process to approve compounds repurposing in rare cancers.

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Conflict of interest

LV reports personal consulting fees from Adaptherapy, is CEO of RESOLVED, has received non-personal fees from Pierre-Fabre and Servier, and a grant from Bristol-Myers Squibb, all outside the submitted work. LV is principal investigator in the Phase 1 unit at CLB of studies with Genmab, Bicycle Therapeutics, Revolution Medicine, Daiichi, Astra Zeneca, Hoffmann La Roche.

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Data availability statement

No data used in this study, only review of the literature unless mentioned

Ethics statement

Each trials and programs presented have their own ethic committee authorisations

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SHORT REPORT

Pioneering the implementation of a precision oncology strategy in Portugal: the Precision Oncology Platform trial

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ABSTRACT

Background and purpose: The Precision Oncology Platform (POP) trial represents the effort of the Portuguese Oncology Institute of Porto (IPO Porto) for joining other leading European institutions in both 'Personalised Cancer Medicine for all EU citizens' (PCM4EU), and 'PRecisIon Cancer MEdicine RepurpOsing SystEm Using Pragmatic Clinical Trials' (PRIME-ROSE) consortia, enabling the development of the Portuguese version of the Drug Rediscovery Protocol (DRUP)-like Clinical Trial (DLCT), based on the experience of the DRUP trial developed in The Netherlands.

Patients/material and methods: The POP trial is a phase II, pragmatic multicentric, non-randomised, open-label study, designed entirely like the other DLCTs. Its primary objective is to describe anti-tumour activity of targeted anticancer drugs in patients with advanced malignancies harbouring actionable molecular alterations. The primary endpoint is disease control rate (DCR). Secondary endpoints encompass treatment-related grade ≥3 adverse events, objective response rate (ORR), duration of response (DOR), progression-free survival (PFS), and overall survival (OS). Exploratory objectives will assess biomarkers, resource use and costs, and patient-reported outcome measures (PROMs).

Interpretation: The POP trial will offer access to innovative treatments for patients without further therapeutic options and provide evidence on efficacy and safety of molecularly-guided treatments. Methodologically, it represents a pioneer approach in Portugal, including a pay-for-performance model embedded in the clinical trial. The POP trial represents a unique opportunity to integrate clinical research within cancer care, pursuing an evidence-based precision oncology strategy, and facilitating its rational and cost-effective implementation into the Portuguese healthcare system.

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Precision oncology; targeted treatment; DRUP-like Clinical Trials; Precision Oncology Platform (POP) trial

Introduction

The implementation of Personalised Cancer Medicine (PCM) represents a transformative shift, promising to overhaul traditional approaches to cancer care. This paradigm leverages targeted therapies to benefit patients whose tumours exhibit specific molecular traits. Increasing knowledge on cancer hallmarks has precipitated the development of innovative treatments, typically relying on tumour molecular profiling and predictive biomarkers to guide therapeutic allocations. Targeted drugs pipeline is rapidly expanding, with accelerated clinical development and regulatory approvals. However, granting of agnostic indications remains constrained, particularly within Europe, where concomitant reimbursement issues often limit access to novel treatments.

Dissemination of PCM poses significant challenges. Widespread expertise in interpretation of complex genomic reports, constituting the baseline for rational use of targeted agents, is lacking. Often, such targeted treatments are marketed drugs prescribed off-label or investigational agents available only through clinical trials. Off-label prescription raises concerns due to unproven efficacy in many cases, as well as effectiveness and safety data on its use are generally not systematically recorded and analysed in routine clinical practice. Unequal access is also a concern because of high cost of new targeted agents and reimbursement issues, contributing to heterogeneity

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in PCM implementation. Expanded/managed access and compassionate use programmes are very limited, not offering sustainable treatment options, and often lack systematic collection of evidence. Based on the experience of the *Drug Rediscovery Protocol* (DRUP) trial developed in The Netherlands, several European institutions have decided to implement *DRUP-like Clinical Trials (DLCTs)*. Nordic countries were the first to open parallel national protocols (IMPRESS-Norway, ProTarget, FINPROVE, MEGALIT). Subsequently, several European partners established a network that will facilitate the exchange of data between the trials, and joined the consortia *Personalised Cancer Medicine for all EU citizens* – PCM4EU [1] and the *PRecisIon Cancer MEdicine RepurpOsing SystEm Using Pragmatic Clinical Trials* (PRIME-ROSE) [2].

The main objective of DLCTs is to assess the efficacy and toxicity of commercially available targeted anticancer drugs for treatment of advanced cancer disclosing potentially actionable alterations. These projects are expected to generate clinical evidence and address the effectiveness of PCM strategies, promoting evidence-based treatment interventions that improve outcomes in cancer care. Through harmonisation and collaboration, the consortia will enable expedited evidence generation for rare mutations/tumour types, due to simultaneous data collection. Additionally, these consortia will cooperate with regulators, policymakers, payers, healthcare providers, and patient advocacy groups to implement evidence based PCM in routine practice, facilitating the rational and cost-effective implementation of the results into the healthcare systems.

The *Precision Oncology Platform* (POP) trial represents the effort of the Portuguese Oncology Institute of Porto (IPO Porto) in joining other leading European institutions in both PCM4EU and PRIME-ROSE consortia, enabling the development of the Portuguese version of DLCTs. IPO Porto is a national reference centre in clinical research, which has been strengthened since the creation of the first Early Phase Clinical Trials Unit dedicated to oncology in Portugal. It has been in charge of implementing precision oncology strategies in our comprehensive cancer centre. Considering the increasing accessibility to sequencing technologies, establishing an institutional multidisciplinary Molecular Tumour Board (MTB) was mandatory for interpretation of molecular results and to guide their rational use for clinical decision-making. Concomitantly, IPO Porto pioneered the establishment of a molecular screening programme, designed as a research project, to allow the implementation of a precision oncology strategy. Indeed, IPO Porto's Precision Oncology Program (POP-IPOP) was developed as a single-site, tumour type-agnostic, prospective observational study, aiming to evaluate the feasibility of using molecular profile-based evidence to support individualised cancer therapy for patients with advanced/refractory, rare or hard-to-treat cancers. Overall, this strategy complies with the recommendation for research centres to perform multigene sequencing as part of the mission to accelerate cancer research and drug development, providing patients access to innovation and prospectively collecting data on the use of next-generation sequencing (NGS) in clinical practice [3] One of the main goals of POP-IPOP, through the MTB, is to accelerate patients' inclusion in clinical trials, and reducing the allocation to off-label treatments. Since its implementation in 2021, IPO Porto screening programme has included more than 400 patients, and its regional expansion is planned in the short term. Therefore, the POP trial will be essential to provide additional treatment options for these patients, but also for patients from other hospitals, reaching nationwide coverage.

Methods

Study design and POP trial state-of-the-art

The POP trial is a phase II, pragmatic multicentric, non-randomised, open-label clinical trial designed to evaluate the efficacy and safety of off-label use of commercially available targeted anticancer drugs. The design of this trial is entirely similar to other DLCTs, described elsewhere [4–6], with just a few local specificities.

The study incorporates three subsequent stages, opening according to the success of current cohorts.

The POP trial protocol has already been presented and discussed with both Pharma companies, represented by the Portuguese Association of Pharmaceutical Industry (APIFARMA) and the Portuguese Regulatory Authority (INFARMED). It will be submitted for formal approval after negotiation and conclusion of the scientific advice process.

IPO Porto is the sponsor of this trial. Additional recruiting Portuguese centres are expected to be opened.

Study objectives and endpoints

The trial's primary objective is to describe anti-tumour activity of targeted anticancer drugs among patients with advanced malignancies harbouring actionable molecular alterations. The primary endpoint is disease control rate (DCR), measured by the proportion of patients achieving complete response (CR), partial response (PR), or stable disease (SD) 16 weeks post-treatment initiation.

Secondary objectives include further assessing the efficacy and safety of tested drugs, evaluate patient-reported outcome measures (PROMs), and examine health-related quality of life. Secondary endpoints encompass the following: proportion of patients with treatment-related grade ≥3 and serious adverse events; objective response rate (ORR), duration of response (DOR), progression-free survival (PFS), and overall survival (OS).

Exploratory objectives will include immune and metabolic tumour responses, genomic/transcriptomic analysis, circulating tumour DNA assessment or microbiome evaluation. Additionally, description of resource use and related costs, together with PROMs will be performed.

Patient population and cohort assignment

Adult and paediatric patients with advanced or metastatic solid tumours or haematological malignancies will be enrolled. They must show disease progression to standard treatments or have

no acceptable treatment options. Enrolment is contingent upon availability of tumour genomic or protein expression test results, demonstrating a potentially actionable mutation and availability of study drugs.

Key inclusion criteria include: adequate organ function, measurable disease, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2.

The trial assigns patients to specific cohorts defined by their tumour type, molecular profile, and targeted agent.

eDrug-specific study manuals describe drug-specific inclusion and exclusion criteria, dosing, toxicity management, projected risk-benefit assessments and treatment schedules.

Negotiations with several pharmaceutical companies, undertaken through the Portuguese Pharma Association [7], are in their final steps, allowing for an equitable and transparent approach under a common memorandum of understanding.

Statistical considerations

As every other DLCT, the POP trial involves a Simon-like two-stage 'admissible' design [8,9] for assessing targeted anticancer drug efficacy across multiple cohorts. Each cohort, defined by tumour type, molecular variant, and treatment, starts with eight participants in stage I. The absence of responses prompts early cohort cessation, while any positive response leads to a stage II expansion to 24 participants. This methodology balances the need for a minimal sample size against the trial's statistical integrity. Response rates below 10% signal clinical disinterest, whereas 30% or above advocate for further investigation (stage III). The selected monitoring rule yields an 85% power with a 7.8% alpha error rate. If stage II yields at least 5 patient responses, the cohort advances to stage III for validation of clinical benefit rate with 80%–90% statistical power (based on clinical benefit rate observed in stage II). The power and sample size calculation will be performed individually for each cohort proceeding to stage III. The POP trial supports data exchange with parallel DLCTs to ensure a robust sample size enabling reliable analysis of all cohorts.

Collateral research

This trial will enable further research, as collateral studies are envisaged, especially for translational purposes and health technology assessment (HTA). Namely, collaboration with several institutional research groups will be crucial for biomarkers analysis. Participants' biological material will be stored at IPO Porto biobank.

Finally, analyses of performance-based risk-sharing agreements (RSA) embedded in clinical research are planned, aiming to methodologically characterise this innovative strategy, its application, and economic impact in the Portuguese healthcare system.

Discussion

A DLCT implementation represents the best opportunity to integrate clinical research within routine cancer care, pursuing a PCM strategy in Portugal. We emphasise the relevance and innovation of the POP trial based on three main aspects: it offers access to innovative treatments for patients without further treatment options; it provides an evidence-generation platform to inform on efficacy and safety of molecularly-guided treatments, and it allows for inclusion of a pay-for-performance model within the clinical trial design.

Integrating PCM and new technologies into healthcare systems constitutes a challenge and public health policies are required to ensure its rational use. Few countries have implemented structured national policies in Europe [10–13], even if some have developed either some legal framework or national plans [14–19]. In Portugal, a coordinated national strategy for PCM implementation is lacking [20]. The European Federation of Pharmaceutical Industries and Associations (EFPIA) recently developed policy recommendations to improve cancer care through broader access to quality biomarker testing [21]. Indeed, a limitation of POP-trial is that molecular screening is not included within the trial, so patients may only be referred if a potentially actionable alteration was already identified. As the access to comprehensive genome sequencing is heterogeneous, referral might also be heterogeneous, showing that additional efforts are needed to develop a PCM ecosystem in Portugal.

Challenges of PCM are also particularly relevant for HTA, especially concerning agnostic indications. These trials have been termed 'histology-inclusive', as they maintain the tissue-agnostic orientation of precision oncology, without losing the relevance of histologic categorisation [22]. DLCTs have an additional cutting-edge feature: stage III constitutes a pay-for-performance model: after the first 4 months of treatment, supplied by the pharmaceutical companies, drugs are covered, only if clinical benefit is confirmed. This constitutes an 'implementation device' for PCM and aims to expand access to drugs besides generating clinical evidence; such an approach also allows for reframing of healthcare as a 'learning system', re-centred on research and simultaneously providing inputs to implementation of healthcare policies in PCM [22].

PCM value assessment framework still needs to build consensus among multiple perspectives, fostering procedures and measures of value aspects [23]. The POP trial's findings will contribute to a dialogue that extends beyond the oncology domain [24], addressing broader questions around drug repurposing, dynamic treatment guidelines, and the economic viability of targeted therapeutics, to guarantee sustainability of healthcare systems. We believe in a patient-centred approach, promoting access to cutting-edge technologies, integrated into an evidence-based context, supporting evidence generation strategies, both from clinical trials and real-world evidence (RWE).

From a value-based perspective, RSA strategies are considered promising tools, enabling outcome-based coverage/reimbursement [25,26]. The POP trial, by including Stage III, represents, to the best of our knowledge, a pioneering approach in Portugal. Indeed, establishing an RSA embedded in a clinical trial represents an innovative strategy, even from a methodological point of view. In our perspective, clinical research should approximate to earlier HTA, addressing the

current gaps in access to medicines, and such type of innovative features of RSA included in clinical trials may represent a possible strategy in this regard. This vision is consistent with the new HTA Regulation [27] and EUnetHTA21 initiative [28] aiming at harmonisation of HTA across Europe and improving equity and access to innovative medicines.

Future trends are expected to evolve towards integrating patient-centred clinical research in a tailored approach. Thus, we believe that the POP trial will become the catalyst for implementing PCM in Portugal, aiming to produce evidence and accelerate drug development, allowing rational and sustainable use of molecular-guided therapies.

Conflict of interest

The authors report there are no conflict of interests to declare.

Author contributions

BM: Conceptualisation; Writing – Original Draft; Writing – Review & Editing.
JA: Conceptualisation; Writing – Original Draft; Writing – Review & Editing.
JD: Conceptualisation; Validation; Writing – Review & Editing.
JO and RH Conceptualisation; Validation; Writing – Review & Editing; Supervision.

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Data availability statement

Not applicable – no data presented.

Ethics declaration

The POP trial protocol will be submitted for approval to competent regulatory authority and Ethics Committee and will be conducted in full conformance with applicable regulation and the Declaration of Helsinki.

Written informed consent will be obtained from all the participants.

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SHORT REPORT

Application of comprehensive molecular genetic profiling in precision cancer medicine, Hungarian experiences

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ABSTRACT

Recent developments in molecular genetic testing methods (e.g. next-generation sequencing [NGS]-panels) largely accelerated the process of finding the most appropriate targeted therapeutic intervention for cancer patients based on molecularly targetable genetic alterations. In Hungary, a centralized approval system following the recommendation of the National Molecular Tumor Board was launched for the coordination of all aspects of comprehensive genetic profiling (CGP) including patient selection and therapy reimbursement.

Aim: The study aims to evaluate the clinical benefit of CGP in our Comprehensive Cancer Center

Methods and patients: CGP was introduced into our routine clinical practice in 2021. An NGS-based large (> 500 genes) gene panel was used for cases where molecular genetic testing was approved by the National Molecular Tumor Board. From 2021 until August 2023 163 cases were tested. The majority of them were ECOG 0–1 patients with advanced-stage diseases, histologically rare cancer, or cancers with unknown primary tumours.

Results: Seventy-four cases (74 of 163, 45%) had clinically relevant genetic alterations. In 34 patients, the identified variants represented an indication for an approved therapy (approved by the Hungarian authorities, on-label indication), while in 40 cases the recommended therapy did not have an approved indication in Hungary for certain tumour types, but off-label indication could be recommended. Based on our CGP results, 24 patients (24/163; 14.7%) received targeted therapy. Treatment duration was between 1 and 60 months. In total 14 (14/163; 8.5% of the tested cases) patients had a positive clinical response (objective response or stable disease) and were treated for more than 16 weeks.

Interpretation: NGS-based CGP was successfully introduced in our institution and a significant number of patients benefited from comprehensive genetic tests. Our preliminary results can serve as the starting point of Drug Rediscovery Protocol (DRUP) studies.

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Tumour mutation profile; molecular tumour board; targeted therapy

Background

In routine clinical practice various molecular genetic tests are used for the identification of therapeutically actionable genetic variants. Single gene tests, various-sized gene panels, or large-scale genetic analyses evaluating > 500 genes, whole exome, and whole genome sequencing are available. In our routine clinical practice, the next-generation sequencing (NGS) approach that uses a single assay to assess approximately 500 genes and genetic variants including relevant cancer biomarkers, as established in guidelines and clinical trials, for therapy guidance in cancer patients has been introduced in 2021 [1]. We refer to this analysis as 'Comprehensive genomic profiling-CGP'.

The availability of NGS-based comprehensive genetic profiling (CGP) in Europe is not uniform. Some countries have already introduced its use in routine clinical practice while in others it is not utilized. Limited access to molecular pathology, clinical genetics, and genomics expertise are among the reasons behind the latter cases, representing considerable inequalities in oncology care in Europe.

CGP offers important benefits to identify molecular alterations that can be used as a therapeutic target [2]. Using it in routine clinical practice is challenging due to the associated high costs and required expertise [3]. In addition to specialists in

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molecular genetic diagnostics, molecular pathology, clinical genetics, oncology, and bioinformatics, significant infrastructural investments are also needed. The benefit and cost-effectiveness of CGP over smaller targeted gene panels have not yet been unambiguously demonstrated. Indications for CGP, according to the The European Society for Medical Oncology (ESMO) guideline, include patients with rare tumours, cancers with unknown primary (CUP), when the therapeutic options have been exhausted but the patient is still in good condition and tumours where the therapeutic indication is based on genomic instability score (GIS) or high tumour mutational burden [4].

To test the clinical benefit of CGP in Hungary the National Health Insurance Fund of Hungary (Hungarian acronym: NEAK) initiated a nationwide molecular tumour board (MTB).

NEAK is the central agency that manages the National Health Insurance Fund and as the only health-related funding agency of the government in Hungary it reimburses all expenses [5]. At four university centers and the National Institute of Oncology various molecular genetic testing methods were introduced into the clinical practice. Comprehensive, large gene panel testing is available from 2019 at two centers in the National Institute of Oncology and Semmelweis University, and from 2022 at the University of Pécs.

MTBs are heterogeneous and a various-sized group of healthcare professionals whose expertise guarantees the most effective workflow and recommendations for cancer patients regarding therapeutic decisions [6]. There are different MTBs: usually every oncology centre where molecular genetic tests are performed has an MTB. However, their size, their members, and their workload can be significantly different. Some are involved at specialized centers (i.e. oncohematology, pediatric cancer etc.) while others (i.e. large centers, typically working closely with comprehensive cancer centers) cover multiple cancer types [7].

Our current work summarizes the steps of the Hungarian Precision Cancer Medicine project started in 2019 at the National Institute of Oncology, Comprehensive Cancer Center, Budapest supported by the Hungarian authorities in line with two European Union-funded projects: PCM4EU (Personalized Cancer Medicine for all EU citizens) and PRIME-ROSE (Precision Cancer Medicine Repurposing System Using Pragmatic Clinical Trials) aim to improve the implementation of molecular genetic test results in direct patient care.

Methods

Initiation of the national molecular genetics and rare cancer tumor board

In our institute, NGS-based assays, mainly smaller targeted panels, were implemented in molecular pathology diagnostics in 2019, and parallel, genetic counseling with comprehensive germline genetic testing for cancer patients has been introduced into routine clinical workflow.

Ordering and availability of molecular genetic testing were performed according to the Hungarian law in a bespoke testing pathway. All patients' samples were reviewed by a local pathologist and the type of molecular test was determined by the tumour cell content and size of the sample in addition to the clinical and pathological diagnosis. In some cases, additional immunohistochemical test was performed by our pathologists to confirm the external diagnosis before initiating the molecular tests.

From the end of 2019 the Hungarian Government and Health Insurance Office (NEAK), to help provide nationwide availability to comprehensive molecular genetic tests, approved the formation of the Molecular Genetics and Rare Cancer Tumor Board, referred to as the National Molecular Tumor Board (NMTB). Requests for NGS-based molecular genetic testing are open for all oncological centers and for all cancer patients from the country. The Hungarian NMTB consists of multi-disciplinary and interdisciplinary expert panel members including four pathologists (two experts in molecular pathology and molecular genetic diagnostics), three physicians specialized in clinical and molecular genetic diagnostics, and clinical genetics, four clinical oncologists, two physicians specialized in radiotherapy, one molecular biologist expert in oncohematology. This board is accompanied by one representative of the NEAK who participates in the weekly meetings. The NMTB reviews the anonymous documentation of patients for whom genetic testing is requested. On average 10–15 cases per week are evaluated. Patients should be at clinical status ECOG 0 or 1. The NEAK provides all relevant documentation to the members of NMTB for evaluation through a secure online platform at least 24 h before the meeting. All previous pathology reports (including results obtained with smaller gene panels), clinical data, and previous therapies are reviewed. During the NMTB meeting, a consensus recommendation is issued and sent back to NEAK who transfer this to the treating physician.

The NGS-based molecular genetic testing is reimbursed for cancer patients by the Health Insurance Office. CGP for somatic testing was introduced in 2021 in our institute. Between December 2021 and August 2023, based on the recommendation of the NMTB 163 cases were tested using CGP. All patients have given consent for participating in the study.

Patients and comprehensive molecular genetic profiling

Of the 163 samples, 109 were primary and 54 were metastases. The distribution of sample types was as follows: 1 cell block, 2 cytology smears, 47 biopsy samples, and 113 resection specimens. Blocks were not older than 3 years.

The localization of tumours is presented in Table 1. Soft tissue, urogenital tumours including high-grade tubo-ovarian serous carcinoma (HGSOC) and cancers of the gastrointestinal system (including pancreatic cancers) were the most prevalent tumour types tested. The common tumours including that is breast carcinoma or lung carcinomas are routinely evaluated by smaller NGS gene panels; therefore, these types are underrepresented in this analysis.

Tumour DNA and RNA were extracted from formalin-fixed paraffin-embedded (FFPE) tissue blocks. Hematoxylin-eosin (HE)-stained sections of all samples were reviewed by a

Table 1. Localization of tumours and the number of patients tested by comprehensive genetic profiling.

Tumour type and localisation	Number of cases
Breast cancer	3
CUP (cancer with unknown primary)	6
Gastrointestinal tumours (including pancreatic cancer, $n=4$)	23
Head and neck cancer	6
Lung cancer	7
Soft tissue tumour	39
Thyroid cancer	5
Prostate cancer	5
High-grade serous ovarian cancer (HGSOC)	16
Other tumors of the urogenital system	30
Other tumor types	23

pathologist to estimate the tumour cell content and select the tumorous part for macrodissection. Nucleic acid isolation was performed using either Maxwell RSC DNA/RNA FFPE Kit on Maxwell RSC Instrument (Promega, USA) or MagMAX FFPE DNA/RNA Ultra Kit on the KingFisher Duo Prime purification system (Thermo Fisher Scientific, USA) according to the manufacturer's instruction. DNA and RNA concentrations were measured using a Qubit Fluorometer with Qubit dsDNA HS Assay and Qubit RNA HS Assay Kit (Thermo Fisher Scientific). Before sequencing the quality of DNA samples was determined using the TaqMan RNase P Detection Reagents Kit (Thermo Fisher Scientific) by quantifying the presence of amplifiable DNA molecules. Samples with $\Delta Ct \leq 2$ compared to the control, predicted their suitability for NGS. QC parameters and tumour cell contents are available on request. The average of tumour cell content was 59% and there were 17 cases with less than 30% tumour cell content.

DNA and RNA libraries were prepared separately with 20–40 ng of input amount and constructed by automated library preparation using the Ion Chef Instrument and Oncomine Comprehensive Assay Plus kit (Thermo Fisher Scientific). Sequencing was performed on the Ion S5 Plus Sequencer. Parameters used for assessing run quality included key signal > 100, Ion Sphere Particles (ISP) loading > 85%, and usable reads > 40%. Parameters used for assessing DNA sample quality included mean read depth >800×, Median Absolute Pair-wise Difference (MAPD) (for copy number variation [CNV] calling) < 0.5, deamination score (for tumor mutation burden [TMB] determination) < 20, and uniformity > 90%. RNA quality metrics included total valid mapped reads > 500,000×, and mean read length > 40 base pairs.

Sequencing data were analyzed using the Torrent Suite Software and Ion Reporter Software on the Torrent Server for automated sequencing data alignment and analysis. Base calling, alignments, and run quality control were performed using the Torrent Suite™ Software v5.18.1. Variant calling, annotation, and assessing TMB, microsatellite instability score (MSI), and homologous recombination (HRD) with the GIS were calculated by Ion Reporter Software 5.20 Workflow Version:3.1.

To make a CNV call the following criteria must be met: MAPD < 0.4, CNV ratio for a copy number gain must be > 2, $P < 10^{-5}$, CNV ratio for a copy number loss must be < 0.85.

A sample-level MSI score is calculated with 76 individual MSI marker's scores. The overall score is used to determine the MSI status of the sample. In case of MSI-H tumors this score is ≥ 18.

The genomic instability metric (GIM) or genomic instability status (GIS) is the same as HRD score. It is a numeric value between 0 and 100 that summarizes unbalanced copy number changes that comes from loss of heterozygosity (LOH), large-scale transitions (LST), and telomeric allelic imbalance (TAI) using genomic segmentation analysis. Higher GIM values correlate with the observation of more genomic instability in the sample. The cutoff value was set for patients with high-grade serous ovarian carcinoma validated on clinical data.

We used the The American College of Medical Genetics and Genomics classification system for variant interpretation by applying online databases (Clinvar, Varsome, Franklin).

Results

In 152 of 163 cases, all QC parameters were appropriate for performing the analysis. In 6 of 163 cases detection of fusion transcripts failed and in 6 cases the determination of TMB failed. The determination of the LST and telomeric allelic imbalance (TAI) indices and GIS could not be determined in eight samples.

Out of the 163 cases tested, 74 cases (45%) had actionable genetic variants. In 34 patients, the identified variants represented an indication for an approved therapy (on-label group), while 40 cases represented an off-label indication in Hungary for the actual tumor type. Off-label indication in our practice means that there is an available The Food and Drug Administration (FDA) and European Medicines Agency (EMA)-approved drug for certain genetic alteration but due to national decisions for certain tumours types is not reimbursed in Hungary, and additionally, the ESCAT level III-IV therapies are also included in this group. In these cases, individual permission approved by the NEAK was required to start the therapy.

The distribution of genetic alterations representing therapeutical indications is summarized in Figure 1. High TMB followed by copy number alterations of *BRCA1/2* genes were the most common findings which indicate on-label therapeutical indication (Table 2).

Regarding tumour types, the highest percentage of genetic alterations with therapeutical targets were identified in breast, lung and prostate carcinomas; however, in these tumour types low number of cases were evaluated. Immunotherapy is approved in Hungary based on the TMB score besides PD-L1 expression. PARP inhibitor therapy is approved for HGSOC, prostate and pancreatic cancer based on alterations of *BRCA1/2* genes or HRD index (Table 3).

NGS-based tests containing a smaller number of genes are also routinely used in our institute for cancer types where approved therapies rely on genetic alterations covered by these panels (such as breast, HGSOC, lung, prostate cancer, colon, and endometrial cancer). During the same period, 1338 smaller

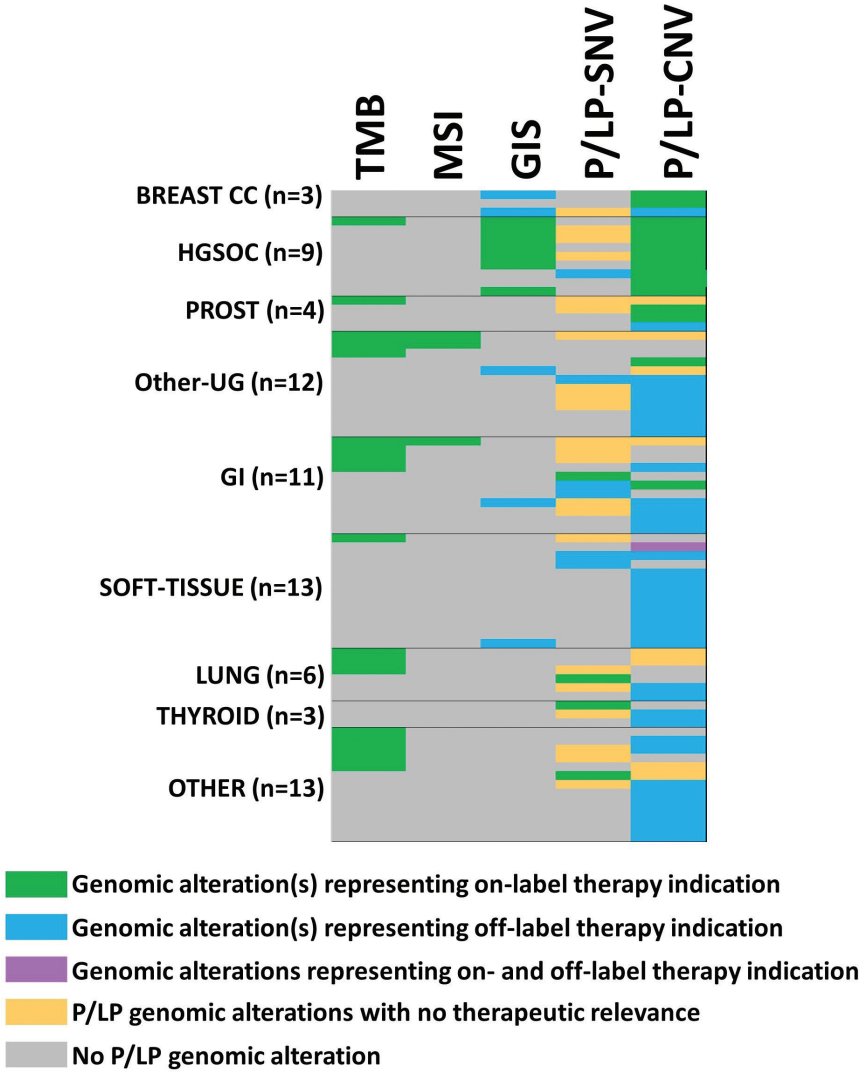

Figure 1. Genetic alterations representing therapeutical indication identified using CPG. Green labels indicate the main P/LP genetic alteration representing an indication for on-label therapy (there can be more than one genetic alteration representing an indication for treatment in the same person). Blue color used for P/LP genetic alteration represents an indication for off-label therapy. Orange color is used for P/LP genetic alteration representing no therapy indication while grey color shows cases without any P/LP variants. The lilac color indicated that there were two genetic alterations; one with on-label (CDK4 CNV), and one with off-label (BRCA2 CNV) indication. P/LP: pathogenic and likely pathogenic variants called by ACMG criteria. CNV: copy number variation.

targeted gene panel tests and 346 somatic *BRCA1/2* tests were performed based on the local decision of molecular pathologists, pathologists, and clinical oncologists. These decisions are made within the organ-specific oncoteams routinely performed at our institute. Using the two smaller targeted panels, of 1338 tested cases 488 (36.5%) cases had a genetic variant that could represent an indication for targeted therapy. In 422 (31.5%) cases the ESCAT (ESMO Scale for Clinical Actionability of Molecular Targets) level I, in 17 cases ESCAT level II, in 43 cases ESCAT level III, and in 6 cases ESCAT level IV therapeutic indications were identified. Of the 346 cases, somatic pathogenic mutations in one of the *BRCA1/2* genes were identified in 97 cases, which represents 28% of all tumours tested, predominantly HGSOC, breast and prostate cancer.

Regarding therapeutical decisions based on CGP results, in total, 24 patients (24/163; 14.7%;11 out of 34 patients received on-label and 13 out of 40 patients received off-label therapy) received therapy (Table 4). The treatment duration was between 1 and 60 months. In total 14 (14/163; 8.5% of the tested cases) patients had a positive clinical response (objective response or stable disease) and were treated for more than 4 months (16 weeks). This is consistent with previously reported data [8, 9]. Treatments were stopped due to toxicity or disease progression.

Reasons for not receiving the recommended targeted therapy in both groups were rapid disease progression, death, or the unavailability of the suggested therapy.

Discussion

Despite the relatively short period of the study, the identified proportion of patients with actionable genomic alterations and the ones who received therapy based on the CGP result, as well as the response and the disease control rates were consistent with previously published data [10].

Table 2. Genetic alterations associated with high TMB in tumours tested by comprehensive genetic profiling.

Tumour type	Pathogenic variants SNV	LOH	Amplification	Deletion, duplication	Fusion	TMB (Mutations/Mb)
High-grade serous ovarian cancer (HGSOC)	n.d.	BRCA1, BRIP1, CDK12, PALB2, POLD1, POLE, PTEN, RAD51B, RAD51C, RAD51D, RAD54L	n.d.	n.d.	Negative	10.52
Brain metastasis of prostate adenocarcinoma	PALB2 exon 4, p.Met296Ter, c.886delA	PPP2R2A, PTEN	n.d.	n.d.	TMPRSS2 – ERG	11.39
Endometrial adenocarcinoma	MSH6 exon 7: c.35571G > C, APC exon 7: p.Arg232Ter, c.694C > T, ATM exon 35: p.Arg1730Ter, c.5188C > T, PTEN exon 5: p.Arg130Ter, c.388C > T, PTEN exon 7: p.Pro246Leu, c.737C > T, ERBB2 exon 17: p.Arg678Gln, c.2033G > A	n.d.	MYC	HLA-A, HLA-B, CDKN2A, ERAP2	Negative	31.41
Endometrial carcinoma metastasis	n.d.	n.d.	n.d.	del: CDKN2A, HLA-B, HLA-A, ERAP2	Negative	35.76
Clear cell ovarian carcinoma	n.d.	n.d.	n.d.	n.d.	Negative	24.82
Adenocarcinoma of the transverse colon	KRAS exon 4: p.Ala146Thr, c.436G > A	n.d.	n.d.	n.d.	Negative	18.97
Colorectal adenocarcinoma	APC exon 16, p.Tyr935Ter, c.2805C > A	n.d.	n.d.	del: NCOR1, CDKN2A, ERAP2	Negative	44.38
Colorectal adenocarcinoma	POLE exon 9: p.Pro286Arg, c.857C > G	n.d.	n.d.	del: CDKN2A	Negative	112.07
Oesophageal adenocarcinoma	not detected	BRCA2 LOH 13q13		BRCA1 exon2–18 duplication	Negative	13.29
Cutaneous angiosarcoma	HRAS exon 3: p.Gln61Leu, c.182A > T			del: CDKN2A, HLA-B	Negative	12.43
Lung adenocarcinoma	not detected	POLD1	MET, FAM135B ; MYC	n.d.	Failed	13.32
Lung large cell neuroendocrine carcinoma	not detected	CHEK2, NBN, POLD1, PPP2R2A, PTEN	MYC	n.d.	Negative	21.01
Lung poorly differentiated carcinoma with neuroendocrine differentiation	LP POLE exon42: p. Gly1923Cys, c.5767G > T,	n.d.	n.d.	n.d.	Negative	32.8
Parathyroid carcinoma	n.d.	n.d.	n.d.	del: CDKN2A, HLA-A, ERAP2	Negative	10.43
High-grade neuroendocrine carcinoma lymph node metastasis	n.d.	BRCA2, BARD1, BLM, CHEK2, FANCL, PALB2, PTEN, RAD51B,	KIT, PDGFRA	n.d.	Negative	16.16
Retroperitoneal high-grade neuroendocrine carcinoma	ATM exon 43: p.Tyr2100Ter, c.6300C > A,	BRCA2	MDM2; DDR2, NFE2L2	n.d.	Negative	87.21
Melanoma metastasis	NRAS exon 3: p.Gln61Leu, c.182A > T,	n.d.	n.d.	n.d.	Negative	39.62
Glioblastoma	n.d.	n.d.	EGFR, PDGFRA	del: PDIA3, MGA, RAD51, TCF7L2, SUFU, CYP2C9, PTEN, ARID5B, MAPK8, GATA3, LARP4B, CDKN2B, CDKN2A, MTAP, HLA-B, HLA-A, EPHA2, SPEN, PGD,	Negative	10.46

n.d.: not detected; del: deletion; TMB: tumor mutation burden.

Table 3. Number and percentage of cases by tumour type harboring therapeutically actionable genetic alterations identified by comprehensive genetic profiling.

Tumor type and localisation	Number of cases tested	Number of cases with targetable genetic alterations	Percentage of targetable genetic alterations (%)
Breast cancer	3	3	100
CUP (cancer with unknown primary)	6	0	0
Gastrointestinal tumours	23	11	48
Head and neck cc.	6	0	0
Lung carcinoma	7	6	85
Soft tissue sarconoma	39	13	33
Thyroid cc.	5	3	60
Prostate cc.	5	4	80
High-grade serous ovarian cancer (HGSOC)	16	9	56
Other tumours of the urogenital system	30	12	40
Other tumour types	23	13	56
Total	163	74	45

Table 4. Therapeutical intervention based on the genetic alterations identified with comprehensive genetic profiling.

Tumour type	Genetic alterations	TMB status	Therapy	Treatment duration (month)	and reason of termination
On-label therapy					
Metastasis of HGSOC	BRCA1 p.Glu23ValfsTer17, c.68_69delAG, ^{X}	low	PARP inhibitor	14	Progression
Colon carcinoma	KRAS p.Ala146Thr, c.436G > A	high^{X}	Immunotherapy	60	On therapy
Metastasis of HGSOC	BRCA1 p.Gln1604AsnfsTer2, c.4806delT^{X} and LOH of BRCA1, BRCA2, BARD1, BRIP1, POLD1, POLE	low	PARP inhibitor	6	Progression
HGSOC	LOH: BRCA1^{X}, BRIP1, CDK12, PALB2, POLD1, POLE, PTEN, RAD51B, RAD51C, RAD51D, RAD54L	failed	PARP inhibitor	5	Toxicity
Lung carcinoma	MET amplification^{X} and LOH of POLD1	high^{X}	MET TKI (Crizotinib) and immunotherapy	4	Progression
Large cell lung neuroendocrine carcinoma	LOH: CHEK2, NBN, POLD1, PPP2R2A, PTEN	high ^{X}	Immunotherapy	8	On therapy
Breast carcinoma	Amplification of BRCA2 exons: 2–11^{X}	low	PARP inhibitor	4	On therapy
Metastasis of melanoma	Deletion of BRCA2 exons 15–16 and exons 19–20; NRAS exon 3: p.Gln61Leu, c.182A > T,	high^{X}	Immunotherapy	4	Toxicity
Ovarian adenocarcinoma	LOH: BRCA1 ^{X}, BRIP1, CDK12, PALB2, POLD1, RAD51C, RAD51D, RAD54L	low	PARP inhibitor	5	On therapy
Rectum carcinoma	APC p.Tyr935Ter, c.2805C > A and del NCOR1, CDKN2A, ERAP2	high^{X}	Immunotherapy	1	Death
Metastasis of endometrial carcinoma	MSH6 exon 9, p.Arg1331Ter, c.3991C > T^{X}, deletions: HLA-B, HLA-A, ERAP2	low	Immunotherapy	1	Death
Off-label therapy					
Cholangiocarcinoma	LOH: BRCA2^{X}, POLE, PPP2R2A	low	PARP inhibitor	11	Progression
Metastasis of breast cancer	dup: BRCA1^{X}, BRCA2^{X} and LOH: ATM, CHEK1, PPP2R2A, RAD51B	low	PARP inhibitor	1	Toxicity
Small cell lung cancer	LOH: BRCA2^{X}, CHEK2, PTEN, RAD54L; BRCA1, BRCA2	low	PARP inhibitor	4	Progression
Metastasis of thymoma	LOH: RAD51B^{X}	low	PARP inhibitor	4	On therapy
High-grade sarcoma	LOH: BRCA1, ^{X} BRCA2, ATM, BARD1, CHEK1, CHEK2, PPP2R2A, RAD51B	low	PARP inhibitor	2	Progression
Malignant peripheral nervous sheet tumor (MPNST)	deletion BRCA2 exons 10–27^{X}	low	PARP inhibitor	5	Progression
Testicular embrional carcinoma	amplification MET^{X}	low	cabozantinib	6	Progression
Parathyroid carcinoma	deletion: CDKN2A, HLA-A, ERAP2	high^{X}	immunotherapy	11	On therapy
Leiomyosarcoma (rectosigma)	LOH: BRCA2^{X}, ATM, CHEK1, CHEK2, POLE, PPP2R2A	low	PARP inhibitor	8	Progression
High-grade spindle cell sarcoma	LOH: BRCA1^{X}, BRCA2, ATM, BRIP1, CDK12, CHEK1, NBN, PALB2, POLE, PPP2R2A, PTEN, RAD51B, RAD51C, RAD51D	low	PARP inhibitor	1	Progression
Metastatic germcell tumor	LOH: BRCA1^{X}, ATM, BARD1, BLM, CHEK1, FANCL	low	PARP inhibitor	6	On therapy
Metastasis of leiomyosarcoma	deletion BRCA2 exon 16–20 ^{X}	low	PARP inhibitor	1	Toxicity
Metastasis of postpubertal teratoma	LOH: BARD1^{X}, CHEK2	low	PARP inhibitor	2	Progression

TMB: tumor mutation burden; $^{X:}$ indicates the genetic alteration representing an indication for therapy.

CGP is a valid and important method for the identification of cases with potentially targetable genetic alterations. This is particularly important for patients where the therapeutical options are limited or the identification of the tumour type is challenging (cases with rare cancers or unknown primary).

In addition to CGP, our centre still uses other, smaller gene panels for routine diagnostic testing mostly because of operational considerations including optimal laboratory workflow, sample size, low tumour cell content frequently detected in certain tumour types, shorter turnaround time of the results, and costs. In addition, in many common cancers, all targetable genetic variants are identifiable by these validated and certified assays, and in these cases, only the TMB and GIS evaluation require CGP [11]. For this reason, the number of common tumours with multiple possible targeted therapies was relatively low in our study. In addition, patients who benefit from therapies like: MEK-inhibitors, BRAF-inhibitors, PI3K-inhibitors, and CDK4/6-inhibitors are often identified using smaller gene panels; therefore, these cases are missing from this cohort (data not shown).

A significant proportion (two-thirds) of the samples tested were primary tumors, with rare histology types where no targeted therapies are available. These cases were tested before or during the first progression. The third part tested were metastases and these patients had several lines of therapy. In this group, any potential actionable genetic variants are very important. Our data showed that nine cases tested from metastases received targeted therapy, and half of them had good clinical response (Table 4).

The high proportion of cases showing actionable targets but not having approved therapy represents a constant challenge. Our data underline the need for a rapid decision from the financial body or, where it is not available, from industrial partners to start the recommended therapy as soon as possible. Finding an adequate ongoing clinical trial was not achievable for our cases either due to the late disease stage or limited ongoing trials.

From January 2023 the European Union financed 'Personalized Cancer Medicine for European Citizens' (PCM4EU) project was launched. Our Institute represents Hungary in this project. Our current work is consistent with the main goals of the PCM4EU project (PCM4EU website (pcm4eu.eu). Having in our disposition the complex molecular genetic workflow including CGP along with complex germline testing we can identify patients in whom a Drug Rediscovery Protocol (DRUP) could be initiated [12]. The DRUP-like trials are prospective phase II combined umbrella-basket trials. The selection of patients in these trials is based on the genomic alterations present in their tumours. Patients with advanced cancers receive targeted therapies based on tumour type and molecular alterations relevant to targeted therapy.

Our report is one of the first reports showing a national effort to introduce CGP into clinical practice. The Hungarian health system and its openness to innovation are unique among European countries. Our practice together with DRUP trials provided firm justification for reimbursement of treatments, which are indicated by CGP results [13, 14].

In summary, our NMTB, established in December 2019 is unique in Europe or worldwide because it is coordinated by the health insurance provider of the country. All decisions are based on experts' opinions and the results and recommendations are immediately translated into clinical practice.

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Disclosure statement: the authors have nothing to declare

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Data availability statement

All data are included in the manuscript. Further queries can be directed to the corresponding author.

Ethics declaration

The study was approved by the Scientific and Research Committee of the Medical Research Council of the Ministry of Health, Hungary (BMEÜ/1630-1/2022/EKU) and all patients have given consent for participating in the study.

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SHORT REPORT

Agent orange exposure and prostate cancer risk in the million veteran program

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ABSTRACT

Background: The US government considers veterans to have been exposed to Agent Orange if they served in Vietnam while the carcinogen was in use, and these veterans are often deemed at high risk of prostate cancer (PCa). Here, we assess whether presumed Agent Orange exposure is independently associated with increased risk of any metastatic or fatal PCa in a diverse Veteran cohort still alive in the modern era (at least 2011), when accounting for race/ethnicity, family history, and genetic risk.

Patients and Methods: Participants in the Million Veteran Program (MVP; enrollment began in 2011) who were on active duty during the Vietnam War era (August 1964-April 1975) were included (n = 301,470). Agent Orange exposure was determined using the US government definition. Genetic risk was assessed via a validated polygenic hazard score. Associations with age at diagnosis of any PCa, metastatic PCa, and death from PCa were assessed via Cox proportional hazards models.

Results and Interpretation: On univariable analysis, exposure to Agent Orange was not associated with increased PCa (hazard ratio [HR]: 1.02, 95% confidence interval [CI]: 1.00–1.04, p = 0.06), metastatic PCa (HR: 0.98, 95% CI: 0.91–1.05, p = 0.55), or fatal PCa (HR: 0.94, 95% CI: 0.79–1.09, p = 0.41). When accounting for race/ethnicity and family history, Agent Orange exposure was independently associated with slightly increased risk of PCa (HR: 1.06, 95% CI: 1.04–1.09, $<10^{-6}$) but not with metastatic PCa (HR: 1.07, 95% CI: 0.98–1.15, p = 0.10) or PCa death (HR: 1.02, 95% CI: 0.83–1.23, p = 0.09). Similar results were found when accounting for genetic risk. Agent Orange exposure history may not improve modern PCa risk stratification.

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Agent Orange; prostate cancer; MVP; race/ethnicity; health disparities

Introduction

Agent Orange, a mixture of herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), kerosene, and diesel fuel, was used in the Vietnam War to clear dense vegetation and destroy food crops. A potential association between 2,4-D and 2,4,5-T exposure [1–3] increased the risk of non-Hodgkin lymphoma, soft-tissue sarcoma, and bladder and lung cancers and has been debated since the 1980s [4], though no adequate epidemiological evidence has supported that conclusion [5]. Agent Orange and early formulations of 2,4-D and 2,4,5-T, were contaminated with a dioxin compound known as 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD), which has been classified as a carcinogen since the 1990s. The Agent Orange Act of 1991 defines exposure to include all veterans who served anywhere in Vietnam between January 9, 1962 to May 7,1975; this Federal definition is used to guide current preventive healthcare policies in this population [6–8]. In the 2000s, a potential association was acknowledged between Agent Orange exposure and genitourinary cancers [9]. However, evidence linking Agent Orange exposure to increased PCa risk or associated mortality among Vietnam War Veterans has been limited to small case series [10–16]. These small studies have found Agent Orange to be associated with slightly lower age at PCa diagnosis, higher incidence of Stage IV disease, and lower rates of biochemical control [8, 16].

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We investigated the association between Agent Orange exposure and PCa risk in the VA Million Veteran Program (MVP), a population-based cohort that started enrollment in 2011 with genotyping, long-term follow-up, and linked clinical records for over 870,000 participating US veterans. The MVP is one of the largest and most diverse electronic health record-linked biobanks in the world, with a unique structure that allows for detailed investigation into the interactions between inherited risk and Agent Orange exposure in US veterans [17]. We tested the hypothesis that Agent Orange exposure, using the practical government definition, is associated with PCa outcomes and thus might improve modern PCa risk stratification for early detection strategies. Moreover, as MVP data have the potential to inform future clinical care and clinical trials (e.g. NCT05129605), it is important to understand how Agent Orange exposure might influence results in this population.

Methods

Participants

We obtained data from MVP for individuals recruited from 63 VA Medical Centers across the United States (US) beginning in 2011. All veterans were eligible for participation in MVP. Study participation included consenting to access the participant's electronic health records for research purposes. The MVP received ethical and study protocol approval from the VA Central Institutional Review Board in accordance with the principles outlined in the Declaration of Helsinki. We limited the present study to males on active duty during the Vietnam War era (August 1964–April 1975) (Table 1). We included PCa diagnoses at any point after Vietnam War service, regardless of when the participant enrolled in MVP. At the time of MVP enrollment, 265,146 participants had no known PCa, 22,609 had a non-metastatic PCa diagnosis, and 1,218 had been diagnosed with metastatic PCa.

Potential Agent Orange exposure

Potential exposure to Agent Orange was determined by the VA Compensation & Pension Committee, as recorded in the MVP data core. As per the legal US government definition, veterans who served physically (on land or inland waterways) in Vietnam during periods of Agent Orange use by the US military were considered exposed to Agent Orange (January 9, 1962–May 7, 1975). Information about the intensity (amount and duration) of Agent Orange exposure for each individual is not known, consistent with routine clinical reality.

Clinical data extraction

PCa diagnosis, age at diagnosis, prostate-specific antigen (PSA) tests, and date of last follow-up were retrieved from the VA Corporate Data Warehouse based on ICD codes and VA Central Cancer Registry data. Age at diagnosis of metastatic PCa indicated the age of the participant when diagnosed with either nodal or distant metastases as determined through a validated natural language processing tool [18]. Fatal PCa information was determined from National Death Index. Participants with ICD10 code 'C61' as underlying cause of death were considered to have died from PCa. Family history was recorded as either the presence or absence of one or more first-degree relatives with PCa. Among the participants eligible for analysis, over 99% had received at least one PSA test in the VA system, though the age at testing and frequency of testing were variable, and clinical indications (screening vs. diagnostic workup) are not known.

Genetic risk: Polygenic Hazard Score (PHS290)

Blood sampling, DNA extraction, quality controls, and imputation were conducted by MVP as described previously [9, 14]. The MVP 1.0 genotyping array contains a total of 723,305 variants, enriched for low-frequency variants in African and Hispanic populations and variants associated with diseases common to the VA population [16].

To assess genetic risk, we calculated a previously developed and validated polygenic hazard score using 290 common genetic variants (PHS290) that reliably stratifies men for age-dependent genetic risk of PCa and is associated with PCa, metastatic PCa, and PCa death [18–20]. Details of PHS290 calculation in MVP are described elsewhere [18, 19]. PHS290

Table 1. Participant characteristics for self-reported race/ethnicity groups among MVP participants who served on active duty during the Vietnam War era (August 1964–April 1975).

	All	Self-reported Race/Ethnicity							
		Non-Hispanic White	Black or African American	Hispanic White	Asian	Native American	Pacific Islander	Other	Unknown
Active duty during Vietnam War	301,470	230,506	45,257	11,009	1,915	3,082	1,292	4,155	4,254
	(84,326)	(68,171)	(9,216)	(3,176)	(447)	(860)	(339)	(1,096)	(1,021)
Fatal prostate cancer	795	525	200	27	<10	<10	<10	13	23
	(221)	(155)	(50)	(6)	(1)	(1)	(1)	(2)	(5)
Metastatic prostate cancer	3,828	2,495	1,033	125	23	29	12	47	64
	(1,113)	(818)	(219)	(35)	(4)	(8)	(4)	(13)	(12)
Any prostate cancer	42,569	29,482	10,084	1,278	224	366	152	481	501
	(12,822)	(9,555)	(2,385)	(403)	(45)	(130)	(46)	(135)	(123)

Numbers indicate participants available for analysis. Numbers in parentheses indicate participants with Agent Orange exposure.

performs well in diverse datasets and is independently associated with PCa risk [18, 19].

Cox proportional hazards analysis

We used Cox proportional hazards models to evaluate the association of Agent Orange exposure with three clinical endpoints: age at diagnosis of PCa, age at diagnosis of metastatic PCa, and age at death from PCa. We also analyzed self-reported racial/ethnic subgroups. Participants with both Black race and Hispanic ethnicity were included in a single category for Black or African American race. Where individuals did not meet the endpoint of interest, we censored at age at last follow-up.

To assess for independent association of Agent Orange exposure with PCa endpoints, we used multivariable Cox proportional hazards models with race/ethnicity, family history, and PHS290. For race/ethnicity hazard ratios (HRs), we used Non-Hispanic White as reference. For PHS290, we illustrated the effect size via the HR for the highest 20% versus lowest 20% of genetic risk (HR80/20) and between other strata of PHS290. These percentiles refer to previously defined absolute thresholds of PHS290 [18, 19]. We assessed statistical significance with two-tailed alpha at 0.01.

PSA testing

Screening has been shown in a large, randomized trial to increase PCa incidence and reduce cause-specific mortality [22], raising the possibility that PSA testing may confound any impact of Agent Orange exposure. We ascertained the number of PSA tests each participant underwent and evaluated associations between Agent Orange exposure and number of pre-diagnostic PSA tests (≥2 years prior to PCa diagnosis) via linear regression. Multivariable linear regressions used race/ethnicity, family history, and PHS290 as predictive variables in addition to Agent Orange exposure.

RESULTS

We found 301,470 veterans eligible for this analysis. Median age at PCa diagnosis was 65.3 years [interquartile range (IQR): 61–69]. Median age at last follow-up was 71.3 [68–74].

On univariable analysis, Agent Orange exposure was not associated with increased PCa diagnosis (HR: 1.02, 95% confidence interval [CI]: 1.00–1.04, $p = 0.06$) (Figure 1; Supplemental Table 1). Some statistically significant associations were found in subgroups based on race and ethnicity (Supplemental Table 1). In the Non-Hispanic White group, Agent Orange exposure was associated with increased PCa (HR: 1.08, 95% CI: 1.05–1.10, $p < 10^{-8}$) and metastatic PCa diagnosis (HR: 1.13, 95% CI: 1.03–1.22, $p < 10^{-2}$). A statistically significant association in the opposite direction was observed in the Black or African American group: those with Agent Orange exposure were somewhat less likely to develop PCa (HR: 0.82, 95% CI: 0.71–0.95, $p < 10^{-2}$). No evidence of association with fatal PCa was seen in MVP participants. Cause-specific cumulative incidence curves for PCa were qualitatively similar regardless of Agent Orange exposure status (Figure 1).

When accounting for race/ethnicity and family history, Agent Orange exposure was an independent risk factor for PCa diagnosis (HR: 1.06, 95% CI: 1.04–1.09, $p < 0.05$) but not for metastatic PCa or PCa death. (Table 2). Genetic risk (PHS290) was

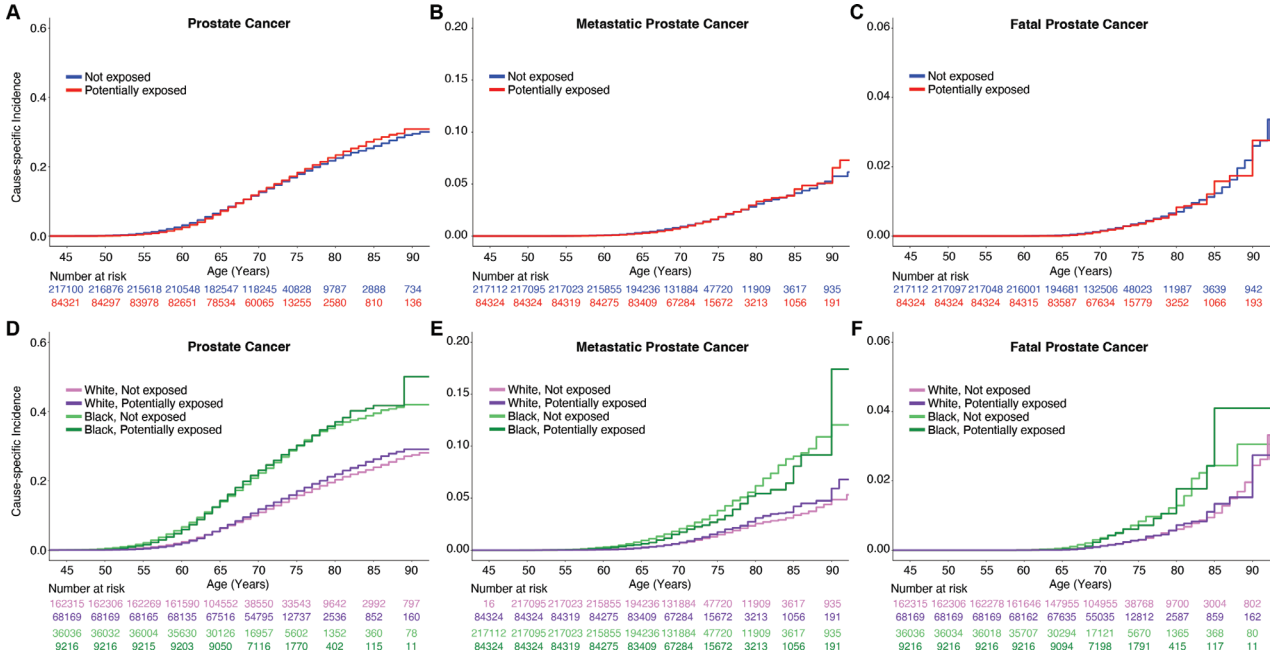

Figure 1. Million Veteran Program (MVP) cause-specific cumulative incidence based on Agent Orange exposure. Cause-specific cumulative incidence among MVP participants on active duty during the Vietnam War, stratified by Agent Orange exposure status (top row) and stratified by self-reported race (bottom row) for (A, D) all prostate cancer, (B, E) metastatic prostate cancer, and (C, F) fatal prostate cancer. 'White' indicates Non-Hispanic White participants, and 'Black' indicates Black and Hispanic Black participants.

Table 2. Multivariable models combining self-reported race/ethnicity, family history, and Agent Orange exposure for three PCa clinical endpoints.

Clinical Endpoint	Self-Reported Race/Ethnicity							Family History	Agent Orange Exposure
	Black or African American	Hispanic White	Asian	Native American	Pacific Islander	Unknown	Other		
Fatal Prostate Cancer	**2.34 [1.87–2.88]****	1.06 [0.53–1.6]	0.26 [0.0–0.8]	0.77 [0.0–1.86]	NA	NA	2.02 [1.04–3.17]	**1.89 [1.45–2.34]***	1.02 [0.83–1.23]
Metastatic Prostate Cancer	**2.49 [2.26–2.72]*****	1.24 [0.97–1.52]	1.03 [0.55–1.53]	0.98 [0.52–1.49]	0.56 [0.0–1.42]	1.78 [0.77–3.13]	1.44 [1.06–1.86]	**1.51 [1.34–1.7]****	1.07 [0.98–1.17]
Prostate Cancer	**2.2 [2.13–2.26]*****	1.02 [0.95–1.1]	0.87 [0.75–1.0]	1.02 [0.9–1.14]	0.89 [0.62–1.18]	0.83 [0.62–1.05]	1.05 [0.95–1.15]	**1.85 [1.79–1.92]*****	**1.06 [1.04–1.09]***

Cox proportional hazards results for association with age at death from PCa, age at diagnosis of metastatic PCa, and age at diagnosis with PCa. *P*-values reported are from multivariable models using self-reported race/ethnicity, family history, and Agent Orange exposure (yes or no). Hazard ratios for race/ethnicity were estimated using Non-Hispanic White as the reference. Hazard ratios for family history were for one or more first-degree relatives diagnosed with prostate cancer. This multivariable analysis was limited to the 213,856 participants who were on active duty during the Vietnam War and for whom family history information was available. Numbers in brackets are 95% confidence intervals. Significant predictors in the multivariable model are indicated by *($p < 0.01$), **($p < 10^{-10}$), and ***($p < 10^{-16}$).

strongly associated with all PCa endpoints, but accounting for this genetic effect had no impact on the association between Agent Orange exposure and PCa diagnosis (Supplemental Table 2). Agent Orange exposure did not differentially modulate PCa risk among men with high genetic risk (PHS290 >80th percentile, as defined previously [21]) or across any PHS290 values (Supplemental Table 3, Supplemental Figure 1).

On univariable and multivariable linear regression analyses in this population, there was no evidence of association between Agent Orange exposure and increased screening. Agent Orange exposure was associated with a statistically significant but small reduction in screening intensity on univariable analysis – 8.3 PSA tests compared to 9 PSA tests for those not exposed. On the other hand, self-reported Black race was associated with increased PSA testing, concordant with guidelines that support stronger consideration of screening for men at higher risk [20] (Supplemental Table 4).

DISCUSSION

In a large, diverse, population-based cohort of US Veterans who served during the Vietnam War and were still alive to enroll in MVP in 2011, Agent Orange exposure was weakly associated with overall PCa, but not metastatic or fatal PCa. Importantly, we present the first multivariable analysis in a population-based cohort to assess whether Agent Orange exposure was an *independent* risk factor for PCa outcomes when accounting for family history, ancestry, and/or genetic risk. Our findings may have pragmatic implications for early detection strategies and suggest the US definition of Agent Orange exposure does not substantially increase risk of morbidity or mortality from PCa, at least for individuals alive today. Also, this study helps inform inclusion criteria for clinical trial enrollment in the VA and sets the foundation to better understand veteran exposures such as burn pits that need to be monitored

Details confirming actual Agent Orange exposure including duration or intensity are not available in MVP or routine clinical practice. Some veterans who physically served in Vietnam while Agent Orange was in use may have had heavy and/or frequent exposure, whereas others may have escaped with little to no exposure. It is possible that intense Agent Orange exposure is associated with aggressive PCa, though adequate data will likely never be available to answer this question. The definition of Agent Orange exposure used in this study is also used by the VA Compensation & Pension Committee to address the needs of potentially exposed individuals. Use of this definition estimates associations of the *average* exposure by those veterans serving in Vietnam during use of Agent Orange. Among Veterans surviving to 2011 or later, we can conclude that average Agent Orange exposure among US veterans serving during Vietnam War era has a much smaller effect size than do family history, Black race, or high polygenic risk. On multivariable analysis, potential Agent Orange exposure yielded HRs < 1.10 for all PCa endpoints underscoring the fact that these statistical associations are not likely clinically meaningful, whereas HRs for metastatic PCa were 1.37 for family history, 1.97 for Black race, and 4.42 for individuals with high versus low polygenic risk (PHS290). Notably, effects may be underestimated as our study focused on veterans who were alive for MVP enrollment in 2011 and did not include veterans who may have died prior to 2011 from Agent Orange exposure effects.

Statistically significant associations in subgroup analyses of self-reported race/ethnicity were small and in opposite directions (increased risk after Agent Orange exposure for Non-Hispanic White participants and decreased risk for Black or African American participants). We interpret these subgroup findings cautiously. On the whole, there is not a clear and strong association of Agent Orange exposure and poor PCa outcomes in MVP.

This study was conducted using data from MVP, so the results may not be generalizable beyond the VA population.

Potential differences in PCa screening intensity between exposure groups were not completely accounted for, though there was no evidence of increased PSA testing among those exposed to Agent Orange in this study. As sequencing for rare pathogenic mutations was not performed, it was also not possible to assess the impact of Agent Orange exposure on risk arising from, for example, germline *BRCA2* mutations, considering Agent Orange mutates genes and induces chromosomal aberrations.

Author contributions

A.J.L., M.S.P., T.M.S. conceived and designed the analysis; A.J.L., M.S.P. and R.K. performed the analysis; J.A.L., K.M.L., S.L.D., J.M.G. contributed data and analysis tools; A.J.L., M.S.P., and T.M.S. wrote the paper with assistance from A.Y.Z., A.P., B.S.R., H.K.C., A.S.K., M.S.P., R.L.H.

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Disclosure statements

None of the authors have a direct conflict of interest relevant to the subject of this study. More broadly, A.K. reports service on the Data and Safety Monitoring Committee for Bristol Meyers Squib and for Cellvax; he also reports consulting for Janssen, Merck, Bayer, and Blue Earth. A.J.L. reports consulting for MIM Software. T.M.S. reports honoraria from Varian Medical Systems and WebMD; he has an equity interest in CorTechs Labs, Inc. and serves on its Scientific Advisory Board; he has received in-kind research support from GE Healthcare via a research agreement with the University of California San Diego. J.A.L., K.M.L., and S.L.D. report grants from Alnylam Pharmaceuticals, Inc., Astellas Pharma, Inc., AstraZeneca Pharmaceuticals LP, Biodesix, Inc, Celgene Corporation, Cerner Enviza, GSK PLC, IQVIA Inc., Janssen Pharmaceuticals, Inc., Kantar Health, Myriad Genetic Laboratories, Inc., Novartis International AG, Parexel International Corporation through the University of Utah or Western Institute for Veteran Research outside the submitted work. These companies might potentially benefit from the research results. The terms of this arrangement have been reviewed and approved by the University of California San Diego in accordance with its conflict-of-interest policies.

Data availability statement

It is not possible for the authors to directly share the individual-level data that were obtained from the MVP due to constraints stipulated in the informed consent. Anyone wishing to gain access to this data should inquire directly to MVP at MVPLOI@va.gov. The data generated from our analyses are included in the manuscript main text, tables, and figures.

Ethics declarations and trial registry information

The MVP received ethical and study protocol approval from the VA Central Institutional Review Board in accordance with the principles outlined in the Declaration of Helsinki.

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LETTER

Desmoplastic non-infantile ganglioglioma mimicking diffuse leptomeningeal glioneuronal tumor: precision diagnostics and therapeutic implications

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Introduction

Desmoplastic infantile ganglioglioma (DIG)/astrocytoma (DIA) are rare entities representing 0.4% of brain tumors and 1.25% of intracranial tumors in children [1]. These tumors typically present before the age of 24 months with clinical signs such as increased head circumference, bulging of the fontanelles, lethargy and sunset sign. There are few reports of non-infantile cases [2]. However, due to a lack of molecular data, the possibility of misclassification was considered and their true existence has been questioned [1]. In 2016, the diffuse leptomeningeal glioneuronal tumor (DLGNT) was introduced as separate entity into the WHO classification of central nervous system (CNS) tumors [3]. Radiologically, DLGNT typically shows a widespread diffuse leptomeningeal enhancement and thickening along the spinal cord, posterior fossa, brain stem and basal cisterns. In contrast, DIG/DIA presents typically as superficially located, large, contrast-enhancing solid and cystic tumor, where the solid component is frequently dural based. Here, we report a non-infantile patient suffering from a brain tumor with radiological features of a DLGNT, but convincing molecular pathological characteristics of a DIG/DIA.

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Brain tumor; HNRNPDL::BRAF fusion; DIG; neuropathology; precision medicine

Case presentation

A 17-year-old female patient presented to the children's hospital with persisting headache, nausea and dizziness over the last 6 months. Clinical examination revealed a healthy-looking girl with age-appropriate features and intact neurological functions. The magnetic resonance image (MRI) revealed widespread leptomeningeal contrast enhancement, tetraventricular hydrocephalus and a left temporomedial subpial cystic lesion with radiological features suspicious for a DLGNT (see Figure 1A–D). Due to the disseminated character of the disease, complete resection was not possible and an open biopsy of the left temporal lesion was performed. Neuropathological work-up revealed a low-grade neuroepithelial tumor with a desmoplastic leptomeningeal component (see Figure 1E-I for histomorphological and immunohistochemical details).

Based on clinical, histomorphological and immunohistochemical features DLGNT and DIG were considered as main differential diagnoses, although the latter appeared less likely due to the disseminative character and the patient's age. Molecular pathological panel analysis with the Oncomine Childhood Cancer panel (ThermoFisher) revealed no genetic alterations. Subsequently, the publicly funded Infrastructure for Precision Diagnostics – cancer (InPreD Norway) was commissioned for further comprehensive molecular work-up. TruSight Oncology 500 (Illumina) analysis revealed an *HNRNPDL*::*BRAF* fusion. The fusion product was assessed to be in-frame with an intact BRAF-kinase-domain (see Supplementary Figure 1). DNA methylation analysis (Infinium Methylation EPIC v.1.0, Illumina) revealed a matching score of >0.95 with the methylation class 'desmoplastic infantile ganglioglioma/desmoplastic infantile astrocytoma' (Heidelberg brain tumor classifier version 12.8). Chromosome arm 1p deletion, an essential diagnostic criterion for the diagnosis of DLGNT, was

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not detected. Based on these findings the biopsy was diagnosed as desmoplastic non-infantile ganglioglioma.

Due to the presence of a BRAF-fusion, treatment with the MEK inhibitor trametinib was initiated. Shortly after the onset of treatment, the initial symptoms disappeared, but the patient experienced skin toxicity, grade 2, in the form of acneiform dermatitis. The skin rash was controlled with additional antibiotic treatment using lymecyclin. Six months after initiation of trametinib administration an increased hair loss was observed. A noticeable regression of leptomeningeal enhancement was seen in the MRI at 3 months follow-up.

On last follow-up, 6 months after commencing trametinib, the patient has no new symptoms, but experience reduced quality of life due to the side effects of trametinib. MRI is without radiologically detected progression in neither supra- nor infratentorial regions (Figure 1J–M).

Discussion

The integration of molecular findings represents a major leap in CNS tumor diagnostics. Based on tumor entity specific molecular alterations, cases with histomorphological and/ or immunohistochemical variation could be summarized into tumor groups that showed similar clinical behavior and outcome of the patients. Such molecular alterations rely on tumor specific mutations, chromosomal aberrations, gene fusions and methylome

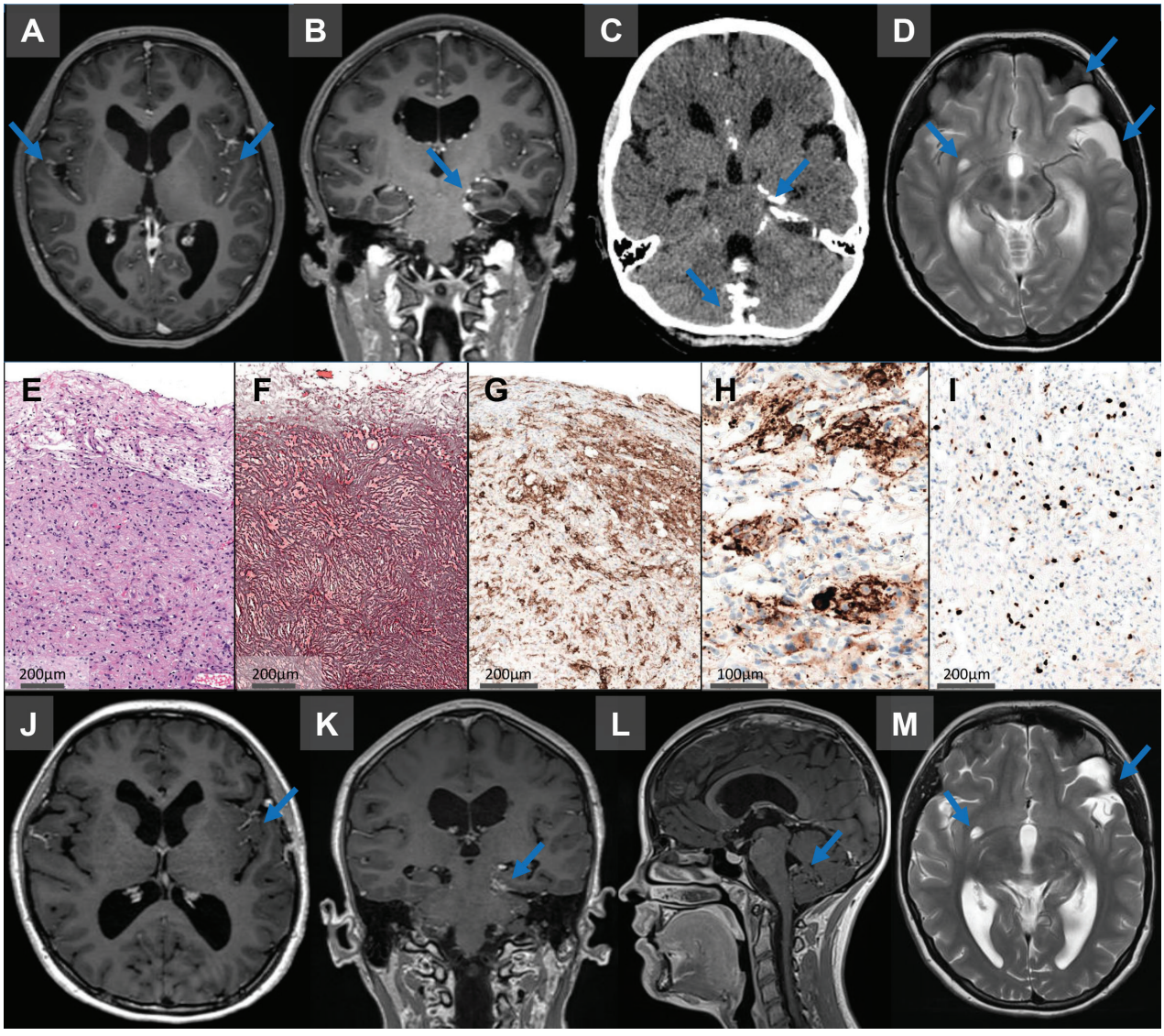

Figure 1. (A–D) MRI caput revealed a widespread leptomeningeal contrast enhancement, partially coinciding with calcifications, including spinal leptomeningeal tumor manifestations. In addition, a left temporomedial subpial cystic lesion with contrast enhancement in the cystic wall and a tetraventricular hydrocephalus was observed. (E–I) Neuropathological findings. Hematoxylin-Eosin staining (E) showed a moderately cellular tumor with a prominent desmoplastic leptomeningeal component, consisting of mainly fibroblast-like, spindle-shaped cells surrounded by reticulin-fibers (F). GFAP (G) highlighted scattered astrocytes within this component and synaptophysin showed a neuronal component with ganglionic differentiation (H). Ki67 immunohistochemistry demonstrated only moderate proliferative activity (I). (J-M) Six months after initiation of medical treatment MRI analysis revealed stable dimensions of the cystic lesion in the left temporal pole. However, there was a partial regression of enhancement observed within the cyst wall, as well as noticeable regression in pathological leptomeningeal enhancement.

profiling. Therefore, the 2021 WHO classification of tumors of the CNS has partially introduced such molecular features as essential for the diagnosis of certain entities [1].

DIG and DLGNT are tumor entities with alterations that typically cause activation of the MAPK-signaling pathway. For DIG, the essential criteria in unresolved cases comprise either a 'methylation profile of DIG/DIA or a BRAF or RAF1 mutation or fusion, occurring in the absence of homozygous deletion of CDKN2A and/or CDKN2B' [1]. For DLGNT the molecular essential diagnostic criteria are chromosome arm 1p deletion and MAPK pathway alteration (mostly *KIAA1549*::*BRAF* fusion) or a methylation profile of DLGNT. *BRAF* fusion proteins, which signal in a dimerized and RAS-independent manner, have an intact *BRAF* kinase domain constitutively active due to replacement of the auto-inhibitory regulatory domain with a 5' partner gene. Targeting downstream with MEK inhibitors represent a rationale to inhibit dimerized forms of *BRAF*-activation. Combined therapy of *BRAF* p.V600E-mutant pediatric low-grade glioma with type I BRAF inhibitor and MEK inhibitor has been approved. However, this combination is not recommended for the treatment of patients with tumors harboring BRAF fusions as type I RAF inhibitors are ineffective in this setting and may paradoxically enhance tumor growth [4].

Typically, the radiological pattern of both entities is rather distinct. The classic radiological features of patients with DIG comprise a superficially located solid and cystic as well as contrast-enhancing lesion. On MRI, the cystic component can be unilocular or multicystic and is T1 hypo- and T2 hyper-intense. The solid component is often dura based and hypointense on T1 and T2 with contrast enhancement.

In comparison, DLGNT shows typically widespread diffuse leptomeningeal enhancement and thickening along the spinal cord. Also, small cystic or nodular T2-hyperintense lesions at the subpial surface of the spinal cord and brain are common. There may be intraparenchymal lesions and there is often an obstructive hydrocephalus and associated periventricular T2-hyperintensity present.

Thus, the current case showed radiological features suggestive for a DLGNT. However, the neuropathological and molecular work-up revealed a desmoplastic component and an *HNRNPDL*::*BRAF* fusion not earlier described in DIG. BRAF-fusions have been detected in different primary brain tumors, particularly in pilocytic astrocytoma (WHO CNS grade 1). However, DNA-methylation analysis showed a clear match with DIG/DIA and there was no deletion of 1p detected. Some patients above the age of 2 years with brain tumors that showed histopathological characteristics of a DIG have been described previously, but these reports were from the pre-genetic era and lack molecular information [2, 5]. True existence of this entity has therefore been questioned [1]. Here, we describe for the first time a glioneuronal tumor in an adolescent patient with molecular and epigenetic findings that fulfill the essential diagnostic criteria for the classification as DIG.

Whereas no molecular alterations were detected using our standard next generation sequencing pipeline, i.e. Oncomine Childhood Cancer panel from ThermoFisher, the diagnostically relevant findings were obtained by using additional molecular analyses such as EPIC methylome analysis and the NGS panel TSO500, with the help of the publicly funded infrastructure for Precision Diagnostics – cancer (InPreD Norway).

This case underlines the need for advanced molecular analyses in the routine setting to allow WHO-conformed diagnoses and identification of possible targets for tailored therapy. It furthermore poses the need of reimbursement, for example as part of the public health care, to establish and maintain state of the art diagnostics.

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Conflict of internet

The authors report that there are no competing interests to declare.

Data availability statement

Data sharing appears not applicable to this article as it could compromise the individual privacy of the described patient. Data that is not subjected to this limitation are available from the corresponding author on reasonable request.

Ethics declaration

Ethical review and approval were not required for this study in accordance with the local legislation and institutional requirements. Informed consent was obtained from the patient included in the study.

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REVIEW ARTICLE

The Tumor Immune Microenvironment in Breast Cancer Progression

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ABSTRACT

Background: The tumor microenvironment significantly influences breast cancer development, progression, and metastasis. Various immune cell populations, including T cells, B cells, NK cells, and myeloid cells exhibit diverse functions in different breast cancer subtypes, contributing to both anti-tumor and pro-tumor activities.

Purpose: This review provides an overview of the predominant immune cell populations in breast cancer subtypes, elucidating their suppressive and prognostic effects. We aim to outline the role of the immune microenvironment from normal breast tissue to invasive cancer and distant metastasis.

Methods: A comprehensive literature review was conducted to analyze the involvement of immune cells throughout breast cancer progression.

Results: In breast cancer, tumors exhibit increased immune cell infiltration compared to normal tissue. Variations exist across subtypes, with higher levels observed in triple-negative and HER2$^+$ tumors are linked to better survival. In contrast, ER$^+$ tumors display lower immune infiltration, associated with poorer outcomes. Furthermore, metastatic sites commonly exhibit a more immunosuppressive microenvironment.

Conclusion: Understanding the complex interaction between tumor and immune cells during breast cancer progression is essential for future research and the development of immune-based strategies. This comprehensive understanding may pave the way for more effective treatment approaches and improved patients outcomes.

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Breast cancer; tumor immune microenvironment; subtypes; progression

Introduction

The role of the immune system is to eliminate pathogens and aberrant cells through immune surveillance. However, this process becomes unsustainable as tumors gradually change the tumor immune microenvironment (TIME) into an immunosuppressive state, evading the host's immune defenses. Tumors employ diverse strategies to escape immune detection, including secretion of immunosuppressive cytokines, downregulation of major histocompatibility complex (MHC) class I, and recruitment of tumor promoting immune cells [1]. The balance between pro- and anti-tumor immune cells emerges as a critical determinant influencing the progression of cancer.

The breast is not an immune-cell rich organ, and breast cancer has not traditionally been recognized as an immunogenic cancer. However, emerging evidence reveals varying degrees of immune cell infiltration across the different breast cancer subtypes. Triple negative breast cancer (TNBC), which lacks expression of human epidermal growth factor 2 (HER2) and the hormonal receptors estrogen and progesterone (ER and PR), and HER2$^+$ breast cancer exhibit higher degree of immunogenicity compared to ER$^+$ tumors. The degree of immune infiltration is hypothesized to reflect the tumor mutational burden, which is higher in TNBC and HER2$^+$ tumors due to genomic instability, leading to increased neoantigen presentation [2].

An in-depth knowledge of the TIME is crucial for understanding tumor progression and in the development of novel targeted therapeutic strategies against breast cancer. In this review, we examine the composition of immune cells and their key roles in the molecular subtypes of breast cancer and through progression from normal breast tissue to metastatic disease.

Immune microenvironment in normal breast tissue

The presence of immune cells in normal breast tissue is relatively scarce. Interestingly, higher immune infiltration is observed in healthy individuals with high risk of developing breast cancer, such as BRCA1 mutation carriers [3]. The immune microenvironment in breast tissue primarily consists of CD8$^+$ T cells, CD68$^+$ macrophages, and CD11$^+$ dendritic cells (DCs) [4–7]. These immune cells are predominantly localized in the breast lobular

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and ductal regions, residing in close proximity to the epithelial cells [4–6]. The CD4⁺ T cells and CD20⁺ B cells are less frequent, and often completely absent from the breast [4]. Recently, a comprehensive study by Kumar et al. [7] using single cell RNA sequencing, identified CD8⁺ and CD4⁺ T cells and M1 macrophages to be the most prevalent immune cells. The CD8⁺ T cells expressed RUNX, indicative of a tissue-resident phenotype. B cells were found in lower numbers, and were dominated by Immunoglobulin G (IgG) and Immunoglobulin A (IgA) producing plasma cells [7].

The breast is an organ undergoing constant change throughout life, influenced by hormonal fluctuations during puberty, the menstrual cycle, and pregnancy. The immune microenvironment is also altered by these hormonal fluctuations [8]. Additionally, age-related alterations are observed in the distribution and localization of immune cells, including decreased B and T cell density in peri-epithelial regions and increased M2 macrophages in the intralobular stroma [9]. These observations support the theory of immunosenescence during aging.

Tumor immune microenvironment in breast cancer

In breast cancer, we see an increased presence of immune cells compared to normal breast tissue; this is summarized in Figure 1. Immune cells of both the lymphoid and myeloid lineage contribute to the dynamic changes seen during tumor progression (Table 1).

Tumor infiltrating lymphocytes in breast cancer subtypes

Tumor infiltrating lymphocytes (TIL) have migrated from the blood stream to the tumor site. TILs encompass a large group of cells, including T cells, B cells, and NK cells. TILs are recognized for their anti-tumor properties, and it is well-established that high numbers of TILs are correlated with a beneficial prognosis in breast cancer [10–12]. High numbers of TILs are also associated with increased likelihood of response to neoadjuvant chemotherapy in all the molecular subtypes [13]. TILs can be classified as stromal (sTIL) or intratumoral (iTIL). Generally sTILs tend to be of higher prevalence than iTILs, and higher sTILs are associated with longer survival in all subtypes [13].

Within the TIL population, T cells with a memory phenotype emerge as the most abundant, playing a pivotal role in the immune response against tumors [14]. Specifically, CD8⁺ T cells serve as effector cells engaged in eradication of tumor cells through recognition of tumor-associated antigens and neoantigens presented by MHC class I. Simultaneously, CD4⁺ T cells provide support to CD8⁺ T cells by secreting a diverse range of effector cytokines.

B cells represent a minority among the TILs, yet their presence holds significance in relation to the formation of tertiary lymphoid structures (TLS). TLS are aggregates of lymphocytes in non-lymphoid tissue. In breast cancer these are found in the stroma and are associated with high-grade tumors [15]. In the context of triple negative breast cancer, these associations are particularly noteworthy, with TLS identified in higher abundance

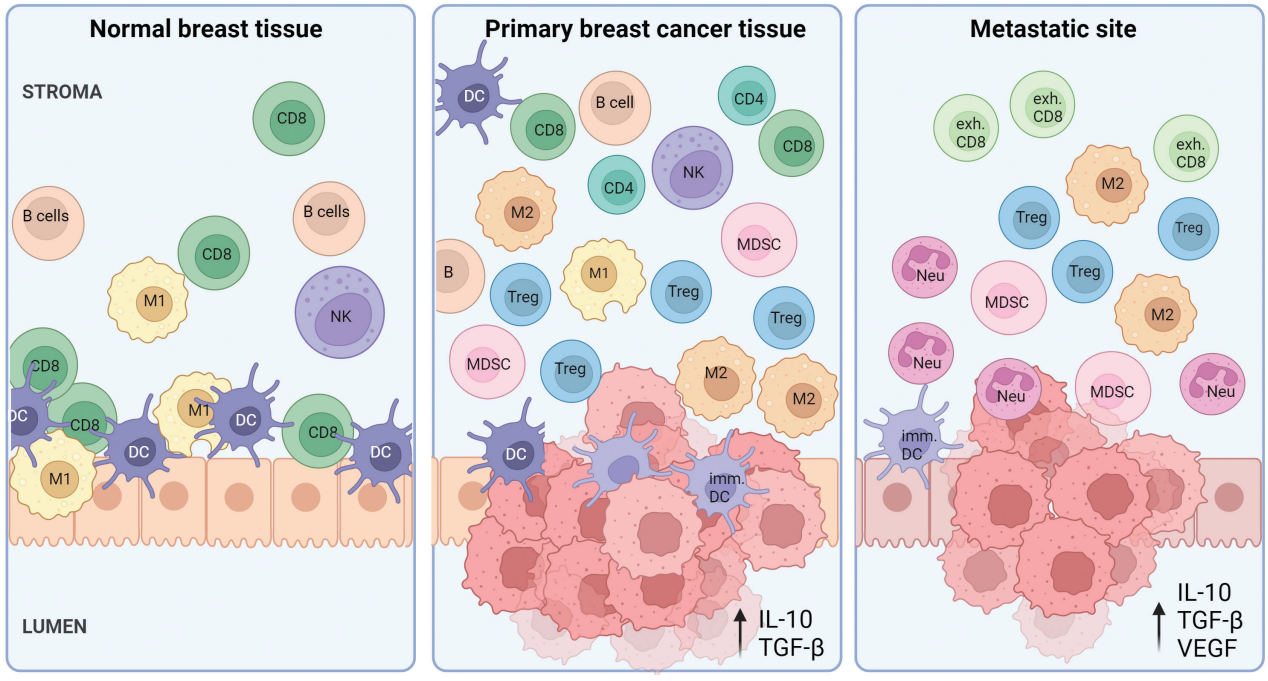

Figure 1. The variation in immune microenvironment from normal breast tissue through the immune escaping invasive cancer to distant metastatic sites. In normal breast tissue, immune cells are located most predominantly within the epithelial regions of the lobules, where CD8 T cells, DCs, NK cells and M1 macrophages are the most dominant cell types. In primary breast tumors, the amount of immune cells increases, where immunosuppressive cells such as Tregs, MDSCs and M2 TAMs aid tumor progression. The TIME in metastatic sites is highly immunosuppressive, including pro-tumor neutrophils, immature DCs and exhausted cytotoxic T cells. CD8=CD8⁺ T cell, exh.CD8=Exhausted CD8⁺ T; Treg=Regulatory T cell; DC=Dendritic cell; imm. DC=Immature DC; M1=M1 macrophage; M2=M2 macrophage; NK cell=Natural killer cell; MDSC=Myeloid-derived suppressor cell; Neu=Neutrophil. Created with BioRender.com.

Table 1. Summary of immune cells, their main markers, and function in the tumor immune microenvironment in breast cancer.

Lineage	Cell type	Main markers	Functions
Lymphoid	B cells	$CD19^+$	Recognize and present tumor antigens to activate T cells, produces tumor-specific antibodies, mediate proinflammatory signaling through the secretion of IFN-γ and direct killing of tumor cells by granzymes. (17–19)
	$CD4^+$ T cells	$CD3^+$ $CD4^+$	Assist $CD8^+$ T cells during the anti-tumor response through the secretion of various cytokines, activate B cells for antibody secretion, and activates macrophages to destroy ingested pathogens. (12,13)
	$CD8^+$ T cells	$CD3^+$ $CD8^+$	Recognize and eliminate cells through the release of membranolytic proteins such as perforin and granzymes (14)
	Regulatory T cells (Tregs)	$CD3^+$ $CD4^+$ $CD25^+$ $FoxP3^+$	Specialized subset of $CD4^+$ T cells involved in the regulation of T and B cell activation. Recruited to the TIME by chemokines and cytokines such as CXCL12, produced by tumor cells and other immunosuppressive cells. Can suppress host immune response by direct cell-cell contacts through CTLA-4 and LAG-3 inhibitory signals, and granzyme/perforin expression and production of immunosuppressive metabolites and cytokines e.g. (IL-10 and TGF- β). Can activate TGF-β secretion by tumor cells, a major cancer immune-escape mechanism. (15–17)
	Natural killer cells (NKs)	$CD56^{bright/dim}$ $CD16^{+/-}$	Monitor and kill abnormal cells. Have the unique ability to recognize and eliminate cells that lack expression of MCH class I, a common evasion strategy for tumor cells. Produce cytokines important for immune surveillance, such as IFN-γ and TNF-α. (18,19)
	Dendritic cells (DCs)	$CD11c^+$ $CD123^+$	Potent antigen presenting cell (APC), initiate adaptive immune responses by engulfing and presenting tumor-specific antigens on MCH class I and II molecules to T cells and producing immunomodulatory signals. Produce type 1 interferon that promote anti-viral and anti-tumor responses. (20,21)
Myeloid	M1 macrophages	$HLA\text{-}DR^+CD68^+iNOS^+$	Eliminate pathogens and tumor cells through direct phagocytosis, activate T cells and NK cells through antigen presentation and secretion of proinflammatory cytokines and chemokines such as TNF-α, IL-1, and CXCL10. (22,23)
	M2 macrophages	$CD68^+CD163^+$	Secrete cytokines such as TGF-β and IL-10 which suppress cytotoxic $CD8^+$ T cells and stimulate Tregs. Promote tumor cell proliferation, angiogenesis, and tissue remodeling through production of growth factors and chemokines such as EGF, FGF, VEGF, and TGF-β. (22,23)
	Myeloid-derived suppressor cells (MDSCs)	$CD11b^+$ $CD14^{-/+}$ $CD15^{-/+}$ $CD33^+$ $HLA\text{-}DR^{-/low}$	Inhibit immune cells such as T cells, DCs, and NK cells, promote angiogenesis and tumor metastasis. Can induce severe anergy of effector immune cells, recruit Tregs at the tumor site, and drive the polarization of M2-like tumor-associated macrophages (TAMs). Inhibit antigen-specific T-cell tolerance, and suppress T-cell responses in an antigen- and neoantigen-specific manner. (6,15,24,25)

VEGF=vascular endothelial growth factor, IFN=interferon, IL=interleukine, TGF=tumor growth factor, TNF=tumor necrosis factor, CTLA-4=cytotoxic T-lymphocyte associated protein 4, LAG-3=lymphocytes activation gene 3

compared to $HER2^+$ and ER^+ subtypes [14]. Tumor infiltrating B cells are associated with an improved clinical outcome in breast cancer [16, 17], although their exact role in anti-tumor activity is not yet fully understood.

Regulatory T cells (Tregs) accumulate in breast cancer tissue compared to normal breast tissue [18], and infiltration of Tregs is correlated with high tumor grade, positive lymph node status and short overall and recurrence-free survival [19]. The prognostic role of Tregs in breast cancer is debated, and some studies have shown opposite results, as reviewed by Saleh and Elkord [19]. Thus, the prognostic effect of Tregs is dependent on the histological grade and molecular subtype.

Natural killer (NK) cells are important cytotoxic cells involved in immune surveillance and direct killing of aberrant cells [20, 21]. In breast cancer, estrogen is well known to have a suppressive effect on NK cells [22, 23]. The presence of NK cells is significantly associated with TILs and Ki-67 index [24]. Because of its killing functions NK cells can be useful in new forms of immunotherapy.

Triple negative breast cancer

Triple negative breast cancer has frequently high infiltration of TILs [25], predominantly $CD8^+$ and $CD4^+$ T cells. B cells [14] and NK cells [24] are also increased in TNBC compared to other subtypes, and the main B cell subpopulation in TNBC is memory B cells, with lower amounts of naïve B cells and plasma cells [26]. Tregs are predominantly found in immune infiltrated TNBC and ER^-HER2^+ subtypes [27, 28]. TNBC with elevated immune infiltration demonstrates enhanced survival rates and increased pathological complete response (pCR) during neoadjuvant therapy [29]. An increased presence of $CD8^+$ T cells is reported to be associated with ER and PR negativity [28, 30], and has favorable prognostic value in ER^- tumors [31]. Surprisingly, while a robust presence of NK cells is associated with a favorable prognosis in ER^+ and $HER2^+$ breast cancer patients, a high presence in TNBC correlates with poor prognosis [32]. This can be explained by the dual role of NK cells as they can also exhibit pro-tumor functions. $CD56^{bright}CD16^{dim}$ NK cells in breast and colon cancers have been found to express the pro-angiogenic factor vascular endothelial growth factor (VEGF), which has a major role in tumor vessel growth and development of an immunosuppressive environment [33, 34]. In a suppressive TIME, NK cells can become dysfunctional due to molecular signals produced by tumor cells and environmental factors such as hypoxia and nutrient deprivation [35].

Table 2. Summary of the presence and prognostic role of the immune cells in the tumor immune microenvironment across the different subtypes of breast cancer.

Presence and prognosis of immune cells	ER$^+$	HER2$^+$	TNBC
Immune cell types with increased presence in breast cancer	Low immune cell infiltration, circ. mDC	TIL, CD8$^+$ T cell, Treg, NK, M2 TAM, circ. pDC	TIL, CD8$^+$ T cell, CD4$^+$ T cell, Memory B cell, Treg, NK, imm. t-pDC, MDSC, M2 TAM
Immune cell types associated with good prognosis	B cell, Treg, circ. mDC, NK	TIL, CD8$^+$ T cell, B cell, NK, circ. pDC	TIL, CD8$^+$ T cell, B cell
Immune cell types associated with poor prognosis	TIL	Treg, M2 TAM	Treg, NK, imm. t-pDC, MDSC, M2 TAM

TIL=tumor infiltrating lymphocytes; NK=natural killer cells; Tregs=regulatory T cells; circ.mDC=circulating myeloid dendritic cells; circ. pDC=circulating plasmacytoid dendritic cells; imm. t-pDC=Immature tumor-infiltrating pDC; MDSCs=myeloid-derived suppressor cells.

HER2$^+$ breast cancer

HER2$^+$ breast cancers are, alongside with TNBC, the subtypes with highest abundance of TILs [28]. The presence of TILs is associated with a favorable prognostic value in both ER$^-$HER2$^+$ and ER$^+$HER2$^+$ tumors [31]. Additionally, in HER2$^+$ breast cancer treated with adjuvant chemotherapy, higher TIL abundance is associated with increased overall survival [13]. An increased presence of CD8$^+$ T cells is associated with favorable prognosis in ER$^-$HER2$^+$ tumors [31]. Conversely, an increased presence of Tregs is associated with HER2 overexpression and decreased overall and progression-free survival [30]. In a spatial context, high CD8$^+$ cell and Treg infiltration in the tumor bed is linked with a decreased survival, while a high CD8$^+$ to Treg ratio in the surrounding area is associated with improved survival [30]. Interestingly, a strong presence of NK cells is associated with positive prognosis in patients with HER2$^+$ subtype, opposite of what is seen in TNBC [24]. Deconvolution methods identified B cell IgG signatures as more strongly associated with pCR and prognosis than TILs in early HER2$^+$ breast cancer [36]. This shows that immune signatures offer valuable insights with potential for predicting treatment responses.

ER$^+$ breast cancer

ER$^+$ tumors exhibit low frequency of TILs. Interestingly, the prognostic impact of TILs is not found to be favorable in this subtype. High TIL infiltration shows adverse prognosis and a shorter overall survival in a neoadjuvant therapy setting [13, 25, 28]. High Treg abundance is linked to lower ER expression [28]. Surprisingly, a high presence of Tregs in ER$^+$ tumors is associated with a better prognosis [30]. NK cells are inversely correlated with ER expression status, and high infiltration is associated with good prognosis in ER$^+$ breast cancers [24].

Tumor infiltrating myeloid cells in breast cancer

Dentritic cells (DCs) are specialized antigen-presenting cells (APC) bridging the innate and adaptive immune responses. There are two distinct types of DCs: plasmacytoid DCs (pDCs) and myeloid DCs (mDCs). pDCs recognize viral infections and produce high levels of interferon type I, whereas mDCs capture, process, and present antigens to T cells [37, 38]. Circulating DCs are more prevalent in breast cancer patients compared to healthy controls [39]. The HER2$^+$ subtype shows the highest amount of circulating pDCs, whereas ER$^+$ subtypes have more circulating mDCs than ER$^-$ subtypes [39]. Lower levels of circulating pDCs are found in patients with later stages of breast cancer [40]. Interestingly, while the presence of circulating pDC is associated with better prognosis, the infiltration into the tumor correlates with adverse outcomes [41]. TNBC exhibits high abundance of both intra-tumor and stromal immature pDC, while ER$^+$ and ER$^+$/HER2$^+$ tumors are dominated by functional mature DCs [42]. Although DCs play a crucial role as anti-tumor cells, the tumor can induce a pro-tumorigenic DC phenotype, leading to dysfunctional and poorly activated DCs [37, 43].

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells with immune regulatory and suppressive functions [32, 44–46]. Recent studies have demonstrated that the release of cytokines, including G-CSF, IL-6, and TGF-β by breast cancer cells influences the expansion and activation of MDSCs, establishing a link between MDSCs and breast cancer progression [45]. An increased abundance of MDSCs is found in TNBC tumors [47]. In TNBC, tumor cells expressing the regulating factor ΔNp63 secrete the chemokines CXCL2 and CCL22, shown to attract MDSCs [47]. Elevated levels of MDSCs in the tumor microenvironment and in circulation are strongly associated with tumor progression and worse overall survival [46]. Furthermore, the level of circulating MDCS is higher in metastatic cancer than non-metastatic cancer [48].

Macrophages are terminally differentiated myeloid cells that can be divided into two categories with opposing actions in the TIME: pro-inflammatory M1 and immunosuppressive M2 tumor associated macrophages (TAMs) [28, 29]. The immunosuppressive M2 TAMs are the most abundant in breast cancer [49], and a high presence is associated with higher tumor grade, ER and PR negativity, and a shorter overall survival, especially in HER2$^+$ and TNBC [50–52].

The precise function and composition of the different immune cells within the different breast cancer subtypes remain unclear. This underscores the challenges in interpreting the roles and functions of the cells in the microenvironment, given the highly heterogeneous nature concerning maturation and differentiation steps. The need for further investigation is evident to unravel the complexities surrounding tumor infiltrating lymphocytes and myeloid cells in breast cancer. A

simplified summary of the immune composition across the molecular subtypes is given in Figure 2, and the presence and prognostic role of the different cell types are summarized in Table 2.

Tumor immune microenvironment in metastatic breast cancer

Many cancer types metastasize to predefined locations in the body, indicating that the spread is not random [53]. The hypothesis of 'seed and soil' was introduced by Paget over a century ago [54], where he proposed that cancer cells (seeds) are thought to thrive and grow in distant sites with favorable conditions (soil), and then ensuring their survival by altering the metastatic environment. The formation of a pre-metastatic niche is created by the primary tumor through several mechanisms including immunosuppression, inflammation, angiogenesis or vascular permeability, lymphangiogenesis, organotropism, and reprogramming [55].

Regional metastasis

Sentinel and the axillary lymph nodes are the lymph nodes located closest to the primary tumor and serve as primary drainage for the breast tissue. Interestingly, the sentinel lymph node, and not the primary tumor, has been suggested to be the first site of tumor–immune interaction [56]. These lymph nodes are the most common sites for metastasis, and approximately 20% of breast cancer patients in Norway have spread to sentinel and regional lymph nodes at the time of diagnosis [57]. Metastatic lymph nodes display a decreased $CD4^+$ to $CD8^+$ T cell ratio [58, 59] and reduced frequency of DCs [59]. Furthermore, various indicators of immunosuppressive environment are noted, including elevated levels of Tregs, MDSCs, and M2 macrophages [60–62]. In metastatic lymph nodes, T cells are discovered to express cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed death receptor 1 (PD-1), and T cell immune receptor with Ig and ITIM domains (TIGIT) and exhibit exhaustion by suppressed TCR signaling [58, 61]

Distant metastasis

Distant metastasis involves tumor cells leaving the primary site and settling in distant organs. While early stage breast cancer has an estimated 5-year survival rate of approximately 95% and regional metastasis of 75%, the survival rate drops drastically to 27% for patients with distant metastasis [63]. The bone is the most frequent site for distant breast cancer metastasis for all subtypes, in particular for $ER^+/HER2^-$ breast cancer, and about 70% of patients with metastatic disease develop bone metastases [64, 65]. The lung and liver are the second most common site of breast cancer metastasis, followed by the brain [66, 67]. The TIME of breast cancer metastasis is highly dependent on the location of the metastasis. By measuring TIL infiltration in secondary lesions from 94 breast cancer patients, Dieci et al. [68] found that TIL levels are generally low (below 5%) in metastatic lesions. In contrast, lung metastases had a median TIL level of approximately 30%.

Bone

Breast cancer is likely predisposed to metastasize to the bone due to the well-vascularized nature of the bone marrow. This

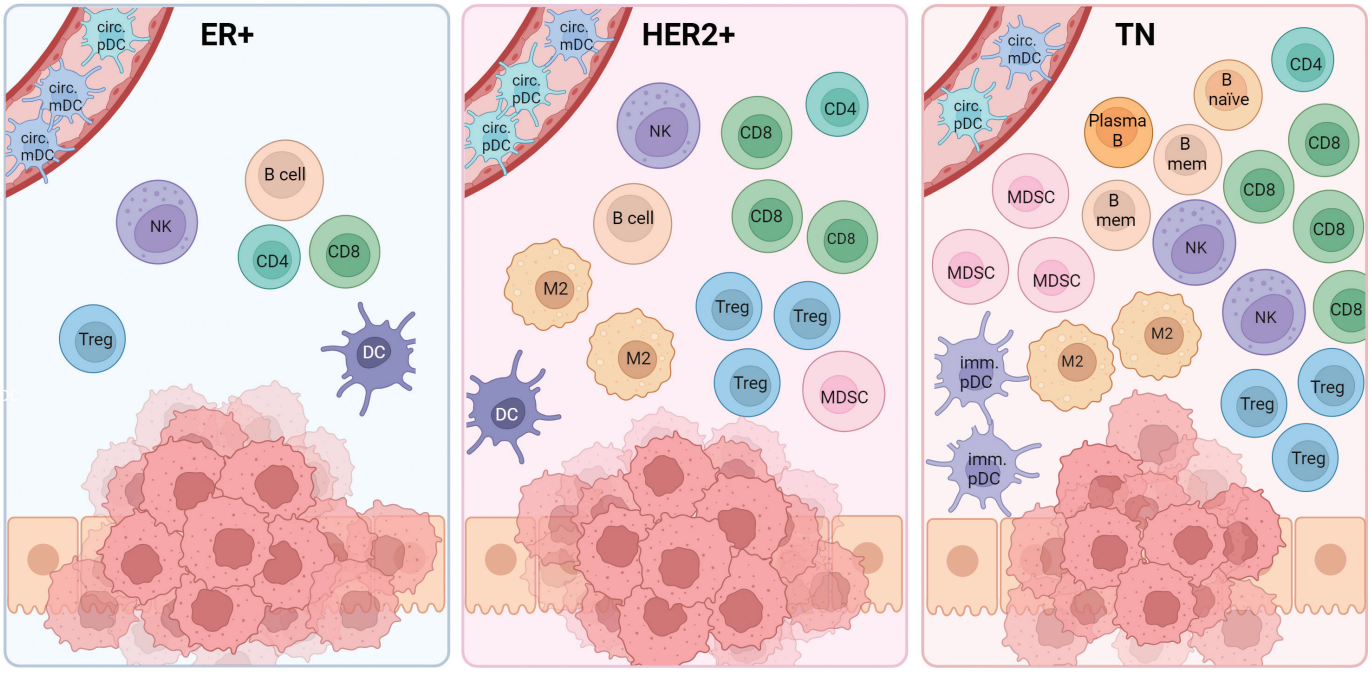

Figure 2. The presence of different immune cells in the molecular subtypes of breast cancer. CD8=CD8 T cell; CD4=CD4 T cell; Treg=Regulatory T cell; DC=Dendritic cell (mature); circ. mDC=Circulating myeloid DC; circ. pDC=Circulating plasmacytoid DC; imm. pDC=Immature pDC; Plasma B=Plasma B cell; B naïve=Naïve B cell; TAM=Tumor-associated macrophage; NK=Natural killer cell; MDSC=Myeloid-derived suppressor cell. Created with BioRender.com.

quality creates a nutrient-rich environment abundant in growth factors and cytokines [69]. By residing in niches in the bone marrow, tumor cells can stay dormant for decades [70]. In this environment, breast cancer cells can interact with mesenchymal stem cells (MSCs), leading to an increased production of Th2 cytokines, recruitment of Tregs, and secretion of MSC-mediated TGF-β1 [71]. These immune modulatory factors contribute to the creation of an immunosuppressive environment, allowing the cancer cells to evade immune detection and elimination by the immune system. Compared to breast lesions, bone marrow metastases show fewer macrophages and an enrichment of neutrophils, indicating an impaired antigen presentation and increased tumor-promoting cytokine secretion [72].

Lung

TNBC commonly metastasize to the lungs [64, 67]. While interacting with the lung stroma, the cancer cells secrete exosomes that stimulates host fibroblasts to create a pre-metastatic microenvironment, and recruit circulating monocytes that differentiate into pro-tumor macrophages [73]. This results in systemic inflammatory cascades leading to neutrophil-mediated promotion of breast cancer metastasis [74].

Liver

HER2$^+$ breast cancer tends to metastasize to the liver [64]. While the liver is rich in immunoreactive cells, it also possesses an immunotolerant microenvironment [75]. Colonization in the liver is facilitated by the secretion of pro-inflammatory cytokines by breast cancer cells, in addition to modulating hepatocytes to increase metastasis [73]. Resident Kupffer cells, liver-specific macrophages, play a role in promoting metastasis by secreting growth factors and recruiting immunosuppressive cells like neutrophils, macrophages, and MDSCs after extravasation [76].

Brain

Both HER2$^+$ and TNBC metastasize to the brain [77–80]. The brain and central nervous system are immune-privileged sites and are partly separated from the immune system by the blood–brain barrier. The predominant immune cell type in the brain is microglia, capable of differentiating into macrophages. The TIME in brain metastasis is identified as immunosuppressive compared to the primary breast tumor [81], with a decrease in CD8$^+$ T cells and M1 macrophages, and minimal presence of B cells [82, 83]. Conversely, M2 macrophages show an opposite trend [81, 83].

Immune checkpoint inhibitors

Immune checkpoints are regulatory pathways in the immune system, and represent important immunotherapeutic targets. Clinical trials on immunotherapy in breast cancer have increased rapidly after the discovery of immune checkpoint inhibitors, and PD1 and its ligand Programmed death receptor ligand 1 (PD-L1) are currently the most studied targets [84]. The interaction between PD-1, present on T cells, and PD-L1 and PD-L2, expressed by APCs and tumor cells, inhibits the cytotoxic effect of the immune cells, promotes T effector cell exhaustion, and promotes the conversion of T effector cells to Tregs [85]. PD-L1 inhibitors in combination with chemotherapy have demonstrated improved progression-free survival for both PD-L1$^+$ (KEYNOTE-355 [86], KEYNOTE-522 [87], IMPASSION130 [88]) and PD-L1$^-$ patients (ALICE [89]). The PD-L1 inhibitor pembrolizumab, in combination with chemotherapy, is approved and used as standard of care in several countries in the treatment of metastatic PD-L1$^+$ TNBC [90–92]. Targeting other immune checkpoint molecules such as T-cell immunoglobulin and mucin domain 3 (TIM-3), TIGIT and Lymphocyte-activation gene 3 (LAG-3) could potentially offer additional novel therapies. Tregs express immune checkpoints, and may be an unintended target for immune checkpoint inhibitors. Blomberg et al. recently discovered that depletion of Tregs in combination with adjuvant checkpoint inhibitors prolonged metastasis-related survival in breast cancer in mice, thus indicating that this could be a potential empowerment of checkpoint therapy [93].

Concluding remarks

The complexity of the tumor infiltrating lymphocytes and myeloid cells, comprising various immune cells, necessitates a deeper exploration of their interplay within the TIME. Technological advancements like single-cell sequencing and multiplexing offer opportunities for more comprehensive analyses, elucidating the dual role of immune cells as both anti-tumor and pro-tumor entities and the interplay between the different cell types. However, numerous aspects remain unknown, emphasizing the need to contextualize immune cell interactions within specific breast cancer subtypes and in various metastatic sites. Integrating emerging technologies and gaining deeper understanding of various immune cell types in breast cancer microenvironment are pivotal for unraveling complexities, refining prognostic and therapeutic strategies tailored to each subtype.

Conflict of interest

The authors report that there are no competing interests to declare.

Data availability

Data sharing is not applicable as no new data generated.

Ethical statement

Due to the nature of the manuscript, ethical considerations do not apply.

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