Topic: Human Tumour Microenvironment Immunoprofiling

All information regarding future IMI Call topics is indicative and subject to change. Final information about future IMI Calls will be communicated after approval by the IMI Governing Board.

Topic details

Action type	Research and Innovation Action (RIA)
Submission & evaluation process	2 Stages

Specific challenges to be addressed

Despite the initial clinical success of cancer immunotherapy with the advent of checkpoint inhibitors and other immune modulating agents, most patients still do not experience a deep and durable response. Numerous trials are currently ongoing exploring combinations of checkpoint inhibitors with established therapies to increase the response rate. Experts in the field are, however, discussing whether all these trials follow a sound scientific rationale. An improved knowledge on the molecular and cellular composition of the tumour microenvironment and better understanding the mechanisms by which the immune system and tumours interact will contribute to more informed decisions on combination therapies and help with developing interventions that would enable better management of the disease and even its cure. Though much has been discovered about the nature of the tumour-host interaction, the basic understanding of how the mechanisms and the different types of immune cells involved in the anti-tumour immune response interact with each other and how they can be monitored and pharmacologically manipulated to better control disease remains somewhat elusive.

To improve therapy, the understanding of the tumour microenvironment needs to evolve:
Firstly, the understanding of **tumour/host interaction on the cellular and molecular level in the absence of therapeutic intervention** needs to improve. Both individual tumours and individual hosts are heterogeneous with respect to the quality and degree of immunity. Understanding the cellular and molecular nature of the tumour microenvironment will (i) help us characterise the ability of a patient's immune system to mount an anti-tumour attack and (ii) provide ideas which pharmacological interventions may support or activate the immune cells to attack the tumour cells.

Secondly and in close alignment with the previous paragraph, one needs to understand **how current therapeutic approaches affect the host/tumour interaction** to **have a baseline** from which to improve the current therapeutic paradigm. Such data could be used to further improve currently available treatments or to develop new potential therapeutic strategies.

A multi-modal approach to assess both the tumour and the host is recommended. Recently developed systems allow for large information-rich data sets to be created that can be mined to gain insights for the development of therapeutic interventions. Furthermore, access to informatics and machine learning tools may lead to the development of clinical and scientific hypotheses that could potentially be validated in the clinic.

This IMI2 topic is designed to generate an information set to help evolve clinical hypothesis generation that will drive the development of new therapeutic interventions for cancer and to identify patient sub-populations that may respond to specific interventions, in particular to immunotherapy. **The proposed topic, for the first time, will assemble a consortium to generate a data set sufficient to gain a meaningful view of the tumour micro-environment. The generation of such a data set is the core activity of this IMI2 topic while the future purposes (improvement of the currently available treatments and development of potential therapeutic strategies) go beyond the frame of this topic.**

Need and opportunity for public-private collaborative research

Given the heterogeneity both in patients' immune systems and tumours, large data sets need to be generated to gain meaningful insights into the tumour microenvironment and the tumour-host interaction both at baseline (without treatment) and under therapy, in particular immunotherapy. This requires access to large numbers of patient samples from numerous clinical centres, collaboration of a number of different partners to analyse them for their molecular and cellular composition. Finally, a collaborative effort is needed to store and integrate patient and sample data according to agreed standards to allow for comparability of data and further analyses. Bioinformatics expertise as well as IT and legal support will be needed. Whilst no single organisation has access to all these resources and expertise (e.g. EFPIA partners: clinical biomarker and drug discovery & development expertise; public partners/clinical centres: patient samples, pathology, histology, etc), all share the same desire and need for a large and standardised dataset on the human Tumour Microenvironment, making this an ideal setting for a public-private partnership.

Scope

The ultimate aim and core activity is **to create a database containing integrated cellular and molecular data from the tumour microenvironment of patients** treated with both targeted and non-targeted therapy, in particular immunotherapy, **as well as key information from patient history and clinical progression**.

- **Core activity (broad profiling):**

  Development of a fully integrated data set of defined immune cell subsets (deliverables (1) and (2)) in samples from patients from specific cancer indications treated with radiotherapy, chemotherapy, targeted therapy and, in particular, targeted immune checkpoint therapy and correlation to the oncogenomic profile of the tumour;

- **Supplemental activities:**
  - In-depth profiling of a subset of samples from patients undergoing immunotherapy using **selected** advanced technologies (deliverable (5));
  - Development of a sustainable open-access, royalty-free and precompetitive database that houses such a data set, including the required privacy settings (deliverables (7-9));

- Generation of a biomarker validation platform to identify and start to characterise potential predictive biomarkers for single-agent and combinatorial immunotherapy trials (deliverable (10)).

Expected key deliverables

1) **Deliverable 1 :**

A data set on presence and spatial distribution of immune cell subtypes (including T cells, NK cells, B-cells, myeloid-derived suppressor cells, macrophages including polarisation markers, neutrophils, dendritic cells, Ki67), using immunohistochemistry (IHC) or immunofluorescence (IF), in surgical specimen (wherever possible) and biopsies with pathologist-validated tumour content, immune infiltrate and invasive front (wherever possible).

IHC or IF measurements should ideally be centralised at one of the academic consortium partners. In case IHC or IF measurements will be performed at multiple sites, a data package needs to be provided demonstrating that such assays can be run using harmonised analysis platforms, reagents and protocols. In any case, validated antibodies should be used and staining and slide scanning should be performed at the same site.

2) **Deliverable 2 :**

RNAseq analysis of all samples as profiled under (1) using ≥ 100M reads per sample.

Deliverables (1) and (2) will be referred to as "Broad profiling" which is regarded as the core activity of the consortium and is expected to consume a considerable part of the resources.

3) **Deliverable 3 :**

Obtaining such data from patients with the following specifications:

a. Pre- and post-treatment tumour samples whenever possible (Pre-treatment: up to six months, preferably immediately prior to treatment and not older than 1 year; post-treatment: should allow informative analyses, e. g. 6-8 weeks after treatment);

b. For immune checkpoint inhibitor (ICI) treatment, pre-treatment samples are mandatory, post-treatment samples are desirable. In case only peripheral samples are available in post-treatment settings, detection of immune cells needs to be performed using suitable methods;

c. For longitudinal studies; collection of samples during the course of therapy (i.e. 1st, line therapy followed by 2nd line therapy, etc.) would be supported and preferred whenever possible;

d. Indications, treatments and envisaged sample numbers:

Indication	
Tumour indication*	**No. of patients envisaged** (please justify deviations from numbers in application)
Lung adenocarcinoma	≥ 600
Head & Neck Cancer	≥ 600
Colorectal Cancer *(with known microsatellite (MS) status)*	≥ 600
ICI failures from different indications	≥ 600
Indication as proposed by academic consortium** (not: melanoma)	≥ 100-600 (flexible; based on the proposal)
All	2500-3000

Treatments	
Type of treatment	**% of patients**
Chemotherapy, radiotherapy, non-ICI targeted therapy	≤ 40% (consortium has to show that large enough sample numbers can be collected for any subgroup to achieve statistical power for broad profiling data set)
ICI therapy (Either post prior therapies or as first line therapy)	≥ 60%
Retrospective versus prospective analysis	
Retrospective (samples max 2ys old, paraffin slides are not sufficient, tumour blocks need to be available)	≤ 30%
Prospective	≥ 70%

*Indications 1-4 are fixed. Lung adenocarcinoma has the highest priority. The consortium should start with this indication and apply any learnings to the other indications.
**This could be a 'classical' tumour indication but could also be a more explorative/subgroup of patients, for example patients who developed cancer under immunosuppressive therapies, e. g. HIV or in a post-transplantation setting.

4) **Deliverable 4 :**

Established and validated workflow for sample quality control, tracking and storage.

5) **Deliverable 5 :**

A "deep profiling" data set for a subset of tumour samples (~50-100 per indication) to address a particular hypothesis, for example patients having undergone or undergoing ICI therapy, with the goal of comparing pre- versus post-treatment samples as derived from, for example:

a. Single cell RNA seq on sorted immune cell population (important);
b. Multi-color flow cytometry, especially of surgical specimen, realised by participating partners that have appropriate capabilities using a standardised panel of markers;
c. Multiplex-IF including a panel of functional immune-related markers;
d. Selected advanced technologies, e. g. CyTOF;
e. Microbiome analysis;
f. ctDNA and ctRNA analysis.

A selected and well-reasoned set of these technologies should be employed; a reasonable and limited part of the budget should be allocated to "Deep profiling" (considerably less than "Broad profiling").

6) **Deliverable 6 :**

For all patients collection and banking of :

a. Blood samples including samples for e.g. paxgene blood-RNA or RNA scope as well as plasma;
b. Faeces.

matched to immuneprofiled tumours to enable future validation of potential predictive biomarkers in peripheral tissue.

7) **Deliverable 7 :**

   A raw data repository with access for all consortium partners.

8) **Deliverable 8 :**

   Software and bioinformatics packages for full data integration and analysis, for example, gene signatures, gene fusions and latest-generation image processing software for analysis of IHC/IF data.

9) **Deliverable 9 :**

   A sustainable database/IT infrastructure allowing for open-access query of data set and long-term housing of database. The data are initially accessible for consortium partners; following data curation, integration and journal publication, the data will be released into the public domain.

10) **Deliverable 10 :**

    Experimental validation packages and classifier signals for potential predictive biomarkers based on the data collected in the consortium.

Wherever possible, synergies with pre-existing platforms, solutions and databases should be realised.

Expected impact

Immunotherapy, as exemplified by therapeutic antibodies neutralising the immune checkpoint PD1, has been shown to provide sustained survival benefit to patients with melanoma, lung, kidney, and bladder cancers. In general, the response rate in these cancer patients to PD1/PD-L1 blockade is about 20 to 30%. Acquired resistance to immune checkpoint blockade is also likely to be observed in some of these responders. While some biomarkers like PD-L1 expression and IFN-gamma gene signature have been able to predict response to PD-1/PD-L1 targeting therapeutics, the mechanisms of resistance, innate and/or acquired, to immune checkpoint blockade in these cancer patients remains largely unknown.

A comprehensive database, profiling immune cells in the tumour microenvironment (TME) of patients that are responsive to immune checkpoint blockade versus those that are not, is generally lacking at the present time and therefore the creation of such a database is the ultimate aim of this IMI2 topic. A searchable database, with integrated tumour genomic information along with matched immune profiles and (immune)therapy outcome, will enable users to identify biological networks involved in and develop biomarkers to predict response to immunotherapy. Maximum impact would be achieved by continued integration of clinical outcome data received after the end of the consortium. The IMI2 topic is expected to be the basis for future significant impacts but these will go beyond the scope and timeframe of the IMI2 topic:

- Identification of novel predictive biomarkers and patient selection strategies and thereby improve clinical response rate to current cancer immunotherapy and other therapeutic regimens in oncology; such discoveries and improvements should enhance clinical and healthcare practice;
- Understanding mechanism(s) of resistance to current immunotherapy, but also other therapy regimens, to enable identification of new therapeutic targets;
- Establishing rational combination immunotherapy strategy (this should strengthen competitiveness and help to address the specific societal challenge of low response rates in cancer patients to current therapies);

- Deriving therapy solutions for patients that are insensitive to immune checkpoint blockade (thus generating a positive impact on European cancer patients' health and wellbeing in the long-term);
- Understanding molecular effects and potential safety liability of immunotherapy.

Overall, the project is consistent with the IMI2 goals of supporting the development of next-generation medicines and treatments for diseases with high unmet medical need as well as treatment biomarkers for diseases clearly linked to clinical relevance.

Potential synergies with existing Consortia

Applicants should take into consideration, while preparing their short proposal, relevant national, European (both research projects as well as research infrastructure initiatives), and non-European initiatives. Synergies and complementarities should be considered in order to incorporate past achievements, available data and lessons learnt where possible, thus avoiding unnecessary overlap and duplication of efforts and funding.

The industry consortium anticipates contributing the following expertise and assets:

- Largely cash contribution (most activities centralised at public partners);
- Work package co-leadership;
- Contribution to database / IT solutions and bioinformatic analyses;
- Contribution to biomarker validation studies.

Indicative duration of the action

The indicative duration of the action is 60 months

Future Project Expansion

Potential applicants must be aware that the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, may publish at a later stage another call for proposals restricted to those projects already selected under this call, in order to enhance and progress their results and achievements by extending their duration and funding. Consortia will be entitled to open to other beneficiaries as they see fit.

As part of a possible future expansion of this IMI2 topic, logical next step activities may be performed that go beyond the time and resource constraints here, e.g. (i) additional tumour indications may be explored, (ii) additional deep-profiling activities may be performed (iii) advanced biomarker testing and validation activities and discovery platforms may be employed and (iv) further IT and data analytics activities may be warranted.

Applicant Consortium

The applicant consortium will be selected on the basis of the submitted short proposals.

The applicant consortium is expected to address **all the research deliverables** (see "Deliverables"), bearing in mind the core activity of the IMI2 topic) and make key contributions to the defined deliverables in synergy with the industry consortium which will join the selected applicant consortium in preparation of the full proposal for stage 2. The size of the applicant consortia should reflect the expertise needed to achieve the proposed objectives within the indicated budget while ensuring the ''manageability" of the consortium as well as efficient and effective team work. Therefore, the number of members of the applicant consortium needs to be thoroughly justified in the proposal and all partners involved should make a significant contribution to the project. **The public partners are expected to carry out the vast majority of the hands-on work,** whereas

EFPIA partners contribute in-kind and cash (see above), so that **work can be carried out centrally with clear streamlined processes.** Steering of the individual work packages and content decisions will be done jointly by the public and private partners. To ensure a rapid and efficient start, it is essential that a translational research infrastructure in oncology with demonstrated collaboration across multiple disciplines (*e.g.* surgeons, trial nurses, medical oncologists, radiologists, pathologists, bioinformaticians, laboratory researchers) is already in place. The consortium is not expected to run dedicated clinical trials.

Specifically, the applicant consortium should be able to demonstrate:

- Access to tumour tissue and matched blood samples from untreated and treated patients (as indicated in the table under expected key deliverables), with fixation/storage appropriate for different analysis methods. It is expected that the entire number of patient samples to be profiled in this project will come from the public consortium. Applicants should demonstrate the feasibility of collecting the outlined number of samples (see Deliverables). EFPIA companies and private partners may contribute additional individual cohorts of patient samples where possible and appropriate;
- Technical expertise to carry out the specified measurements using a harmonised set of platforms, protocols & reagents for all consortium partners;
- Established and validated workflow for sample quality control, tracking and storage. If such processes do not exist yet in the manner necessary to centralise essential steps in the consortium as outlined in the deliverables, the ability to set this up should be shown;
- Experience (as demonstrated by manuscripts/publications/other study reports) on a core set of „deep profiling“ technologies to be carried out on a subset of samples. Some “deep profiling” technologies might be established during the course of the project or could be performed by SMEs;
- Ability to have legal frame (informed patient consent forms = IPCF) in place for full duration of consortium and beyond that allows:
    - Acquisition of samples and experimental & bioinformatics studies outlined in the deliverables;
    - Transfer of raw and processed experimental data as well as relevant data from medical history in anonymised fashion into data repository/database and open access for consortium members and later, the greater public;
    - Maintenance of documentation of IPCFs;
    - Operation under General Data Protection Regulation (EU) 2016/679 (effective May 2018) for European partners, or according to local regulations in case of data from other partners;
    - Adherence to any other national laws and regulations.
- Experience of handling, analysing and integrating large and complex data sets including housing a database;
- To support standardization of data, adherence to the FAIR principles (Findable, Accessible, Interoperable and Reusable), as outlined in the standard starter pack developed by **eTRIKS**: https://zenodo.org/record/50825#.Wa5XC7IjHIV;
- Ability to technically and legally establish and maintain an open-access database beyond consortium frame;
- A plan for aspects related to sustainability, especially ensuring the database remains accessible and facilitating its population with additional clinical outcome data should be proposed. This can include a proposal for options transferring the open access database into an existing structure and should include realistic ideas for long-term financial and operational sustainability of the database;
- Maximum impact would be achieved if collection of clinical outcome data for at least two years beyond consortium frame and integration of the collected data into the database is possible;
- Ability to coordinate a large research initiative and to create a scientific network;
- Ability to involve Patient Advocacy Groups in such projects.

The applicant consortium is expected to set up a governance structure that includes the necessary project management skills suitable for the consortium and activities.
This could be ensured by one of the publicly funded partners, who in this case would need to have significant project management and coordination skills as well as the necessary experience in supporting complex – per size and composition – consortia in IMI/EU funded projects.

The resources allocated should be adequate for the complexity and size of the consortium.

Suggested architecture of the full proposal

In the spirit of the partnership, and to reflect how IMI2 JU call topics are built on identified scientific priorities agreed together with EFPIA beneficiaries, these beneficiaries intend to significantly contribute to the programme and project leadership as well as project financial management.

The final architecture of the full proposal will be defined by the participants in compliance with the IMI2 JU rules and with a view to the achievement of the project objectives. The allocation of a leading role within the consortium will be discussed in the course of the drafting of the full proposal to be submitted at stage 2. To facilitate the formation of the final consortium, until the roles are formally appointed through the consortium agreement, the proposed project leader from among EFPIA beneficiaries shall facilitate an efficient negotiation of project content and required agreements. All beneficiaries are encouraged to discuss the project architecture and governance and the weighting of responsibilities and priorities therein.

The public partners are expected to carry out the vast majority of the hands-on work whereas EFPIA partners contribute in-kind and cash (see above), so that work can be carried out centrally with clear streamlined processes. Steering of the individual work packages and content decisions will be done jointly by the public and private partners. In addition to project leadership, industry partners staff efforts will be largely spent on work packages 4-8, with major involvement of industry partners in work package 7. Further details will be worked out between the industry consortium and the winning public consortium at stage 2.

All work packages will be co-led by EFPIA and public partners and are expected to have adequate autonomy. A lean governance structure should be put in place.

The architecture outlined below for the full proposal is a suggestion. Different innovative project designs are welcome, if properly justified.

Work package 1 – Management & steering, coordination, sustainability planning; project management office

Industry contribution:

- Project leader;
- Coordination across different WPs (including overall scientific and strategic oversight).

Expected Applicant consortium contribution:

- Project coordinator;
- Professional project management expertise (daily operational support with project meetings, reporting and internal communication), e. g. through a project management office;
- See section on Applicant Consortium.

Work package 2 – Communication, Public Relations, and involvement of Patient Advocacy Groups

Industry contribution:

- Communications and patient advocacy expertise.

Expected Applicant consortium contribution:

- Carry out communication on project overall;
- Involve patient advocacy and other groups of interest, e.g. to support patient consent;
- See section on Applicant Consortium.

Work package 3 – Legal aspects

Industry contribution:

- Legal input to support discussions around informed patient consent form & data privacy;
- Enable potential synergies with IMI2 DO->IT consortium.

Expected Applicant consortium contribution:

- Ensure legal frame is compatible with deliverable;
- Implementation of legal frame to allow execution of all the deliverables, e.g. IPCFs, data privacy, data repository and access, etc;
- See section on Applicant Consortium.

Work package 4.1 – Broad profiling (Core activity)

Industry contribution:

- Input and expertise to selection of profiling technologies, antibodies, and immune cell subtypes to be analysed;
- Expertise in selected profiling technologies, image analysis and primary data analysis;
- Oversight of broad profiling activities and results.

Expected Applicant consortium contribution:

- Input and expertise to selection of profiling technologies, antibodies, and immune cell subtypes to be analysed;
- Applicants are expected to carry out all broad profiling activities, including sample taking, staining, slide scanning, RNAseq, and analysis;
- See deliverables 1-3 and section on Applicant Consortium.

NOTE : Profiling costs (consumables, RNA seq etc) for all samples outlined in deliverable 3 are expected to consume a substantial part of the cash budget and resources, which should be outlined in the application.

Work package 4.2 – Deep profiling

Industry contribution:

- Input and expertise to selection of deep profiling technologies;
- Expertise in selected deep profiling technologies and primary data analysis**.**

Expected Applicant consortium contribution:

- Input and expertise to selection of deep profiling technologies;
- Applicants are expected to carry out all deep profiling activities;
- See deliverable 5 and section on Applicant Consortium.

NOTE: A selected and well-reasoned set of technologies should be employed; a reasonable and limited part of the budget and resources should be allocated (considerably less than “Broad profiling”).

Work package 5 – Patients/indications: oversight sample banking and management, QC and ethics

Industry contribution:

- Expertise in sample logistics and quality control;
- Input to process and oversight of validated workflow for sample quality control, tracking and storage/banking (deliverables 4 and 6);
- Oversight of sample logistics, quality control etc.

Expected Applicant consortium contribution:

- Possess or deliver workflows for sample collection, quality control, tracking, storage, banking and maintenance, also linked to legal frame, and implement and carry them out for the project;
- See especially deliverables 4 and 6 and section on Applicant Consortium.

Work package 6 – Biomarker validation

Industry contribution:

- Experimental and technical expertise, pharmacological tool agents;
- Input to idea generation and oversight on biomarker validation (deliverable 10);
- Laboratory and computational approaches related to I/O biomarkers.

Expected Applicant consortium contribution:

- Input to idea generation and execution of biomarker validation (deliverable 10);
- See deliverable (10) and section on Applicant Consortium;

NOTE: A reasonable and limited part of the budget and resources should be allocated.

Work package 7 – Data integration and bioinformatics

Industry contribution:

- Input and oversight of bioinformatics, data integration and statistics support;
- Support / carry out software and bioinformatics packages for data analysis.

Expected Applicant consortium contribution:

- Input to and implementation of software and bioinformatics packages for full data integration and analysis;
- Carry out data analysis;
- See deliverable 8 and section on Applicant Consortium.

Work package 8 – Database and IT infrastructure

Industry contribution:

- Database expertise;
- Input on database infrastructure and testing.

Expected Applicant consortium contribution:

- Implement a raw data repository, upload and maintain data, make data accessible to different consortium members;
- Develop and implement a sustainable database/IT infrastructure as outlined in deliverable 9;
- Carry out the activities for deliverables 7 and 9;
- See deliverables 7 and 9 and section on Applicant Consortium.