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Review

The NANOTUMOR consortium – Towards the Tumor Cell Atlas

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Cancer is a multi-step disease where an initial tumour progresses through critical steps shaping, in most cases, life-threatening secondary foci called metastases. The oncogenic cascade involves genetic, epigenetic, signalling pathways, intracellular trafficking and/or metabolic alterations within cancer cells. In addition, pre-malignant and malignant cells orchestrate complex and dynamic interactions with non-malignant cells and acellular matricial components or secreted factors within the tumour microenvironment that is instrumental in the progression of the disease. As our aptitude to effectively treat cancer mostly depends on our ability to decipher, properly diagnose and impede cancer progression and metastasis formation, full characterisation of molecular complexes and cellular processes at play along the metastasis cascade is crucial. For many years, the scientific community lacked adapted imaging and molecular technologies to accurately dissect, at the highest resolution possible, tumour and stromal cells behaviour within their natural microenvironment. In that context, the NANOTUMOR consortium is a French national multi-disciplinary workforce which aims at a providing a multi-scale characterisation of the oncogenic cascade, from the atomic level to the dynamic organisation of the cell in response to genetic mutations, environmental changes or epigenetic modifications. Ultimately, this program aims at identifying new therapeutic targets using innovative drug design.

Introduction

The cellular organisation and organelle morphology reflect the integrated action of the expressed proteins directed by the genetic and epigenetic program of a given cell type. The cellular ultrastructure is affected by metabolic or pathologic changes and therefore informs us about the physio-pathological state of the cell. Deciphering the links between genetic alteration, cell morphology and disease progression could therefore help to establish a diagnosis and/or determine the stage of a large number of human diseases and in particular in cancer (Rozenblatt-Rosen et al.,

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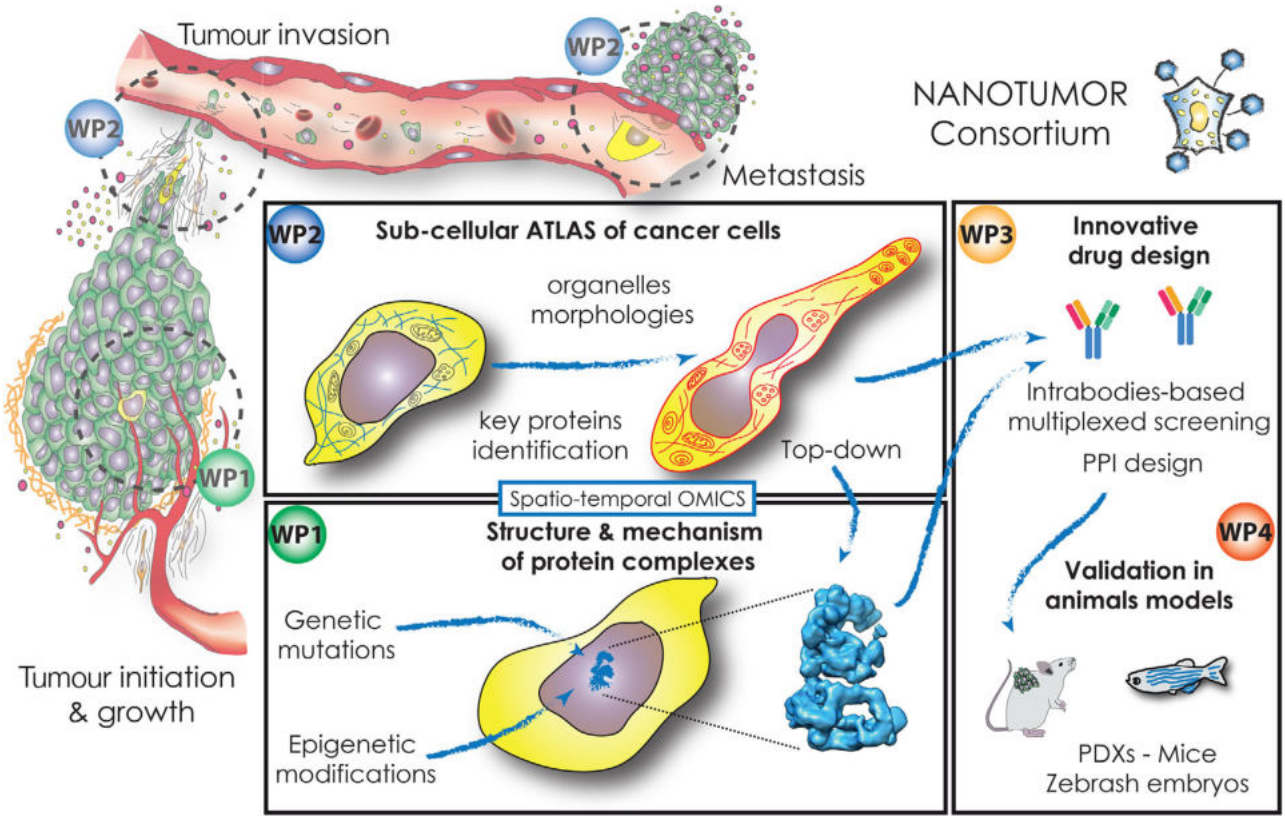
Abbreviations: ATRX, transcriptional regulator part of the SWI/SNF family of chromatin remodeling proteins; Aviesan, French national alliance for life and health sciences; BMSV, French molecular and structural bases of life ITMO; DAXX, death-associated protein 6, H3.3 specific histone chaperone; ELISA, enzyme-linked immunosorbent assay; ES cells, embryonic stem cells; EVs, extracellular vesicles; H2A.2 & H3.3, histone variants; HTAN, human tumor atlas network; ITMO, French multi-agency thematic institute; MS, Mass spectrometry; PPI, protein-protein inhibitor; SWI/SNF, subfamiliy of ATP-dependent chromatin remodeling complexes; WP(s), work-package(s)

Figure 1 | Schematic Representation of the NANOTUMOR Project which aims at a multi-resolution description of the oncogenic cascade from the atomic level to the dynamic organisation of the cell in response to genetic mutations, environmental changes or epigenetic modifications, with the possibility to interfere with these processes with innovative drug design.



2020), which is the focus of the NANOTUMOR consortium (Figure 1). The pathway between one – or a collection of – point mutation(s) identified in a patient affected by cancer to the actual cellular defect is often not straightforward. Indeed, cells behave like multicomponent networks in which the alteration of one or several component(s) may have distal, antagonist or synergic effects which are difficult to predict. Thus, an adequate cure might not be to replace the exact function of the altered protein(s), but to remedy more general downstream effects that may be revealed by cell morphology alterations. Consequently, an atlas of normal and cancer cell morphologies throughout the oncogenic cascade may guide researchers and clinicians towards altered pathways for which treatments exist.

A major goal of the NANOTUMOR Consortium (Figure 1) is thus to provide the nanoscale and molecular maps of cancer cells, at different steps of the metastasis cascade, from tumour initiation to metastasis formation. Our ambition is to provide high-resolution characterisation of subcellular processes and cellular organelles, within tumour cells. Cells are occupied by numerous organelles which carry important cellular functions and are constantly remodelled to exchange molecules and carry out important cellular functions. In addition, being responsible for the viscoelastic properties of any cell (Pu et al., 2016) – and thereby of tumour cells as well –, these organelles are under extreme mechanical/metabolic stress when tumour cells are subjected to high compression or tension forces which they need to cope with during their metastatic journey (Gensbittel et al., 2020; van Bergeijk et al., 2016). Indeed, mechanical forces alter nuclear envelope structure and composition, chromatin organisation and gene expression, which are likely to change the fate of cancer/metastatic cells (reviewed in Gensbittel et al., 2020). A careful

evaluation of the effect of mechanical forces, among other constraints, and metastatic steps on cellular organelles is crucial. Such information would allow to understand their effects on important organelle-carried cellular functions, in addition to identifying organelles and cytoskeletal elements that are responsible for rheological responses and differences. Furthermore, although cancer is a disease that actually covers a heterogeneous combinatorial spectrum of genetic and epigenetic alterations in various micro-environmental contexts, transformed cells are characterised by a handful of common mechanistic behaviours, such as stemness, proliferation and invasion. This leads to the assumption that modules that most likely present converging, significant and interpretable patterns in cancers may extend upwards in scale to include protein complexes, broad cellular processes, and in particular organelles. A major aim of our program is thus to shed light on the alterations of intracellular organelles during cancer progression and to use this higher order modules to delineate mechanisms driving tumorigenesis. We aim at establishing an unprecedented top-down strategy to stratify protein networks driving cancer and exploit key protein interactions and complexes for vulnerabilities of therapeutic relevance. Once protein networks have been identified, we aim at purifying, characterising and determining the atomic structure of molecular complexes involved in the cancer cascade. Recent progresses in molecular biology, mass-spectrometry coupled to cross-linking or single particle cryo-EM opens the possibility to identify major cellular nano-machines in terms of protein composition, post-translational modifications, functional dissection, and determine atomic models of molecular assemblies of low cellular abundance. Such developments could, in the near future, be applied to samples coming from cancer patients but also offer the opportunity to determine a structural framework to explain the mode of action of these complexes, to shed light on their dysfunction in human diseases, and to describe in atomic details key molecular interactions that can be used for innovative drug design. We thus aim at describing the structural alterations and the modifications of the interaction partners induced during cancer disease progression and upon drug resistance. The biochemical and structural characterisation of the cancer-induced alterations of molecular complexes needs to be validated *in vivo*. The structure of key protein–protein interfaces will be a powerful asset for *in silico* drug design and purified complexes will be available for *in vitro* screening and lead optimisation. In conclusion, the overarching objective of the NANOTUMOR project is to reach a higher level of integration and correlate mutations found in specific molecular complexes with the appearance of characteristic cellular morphological traits in order to explain the neoplastic transformations. We anticipate major insights into the underlying tumour biology allowing rational design of antitumour strategies.

NANOTUMOR consortium

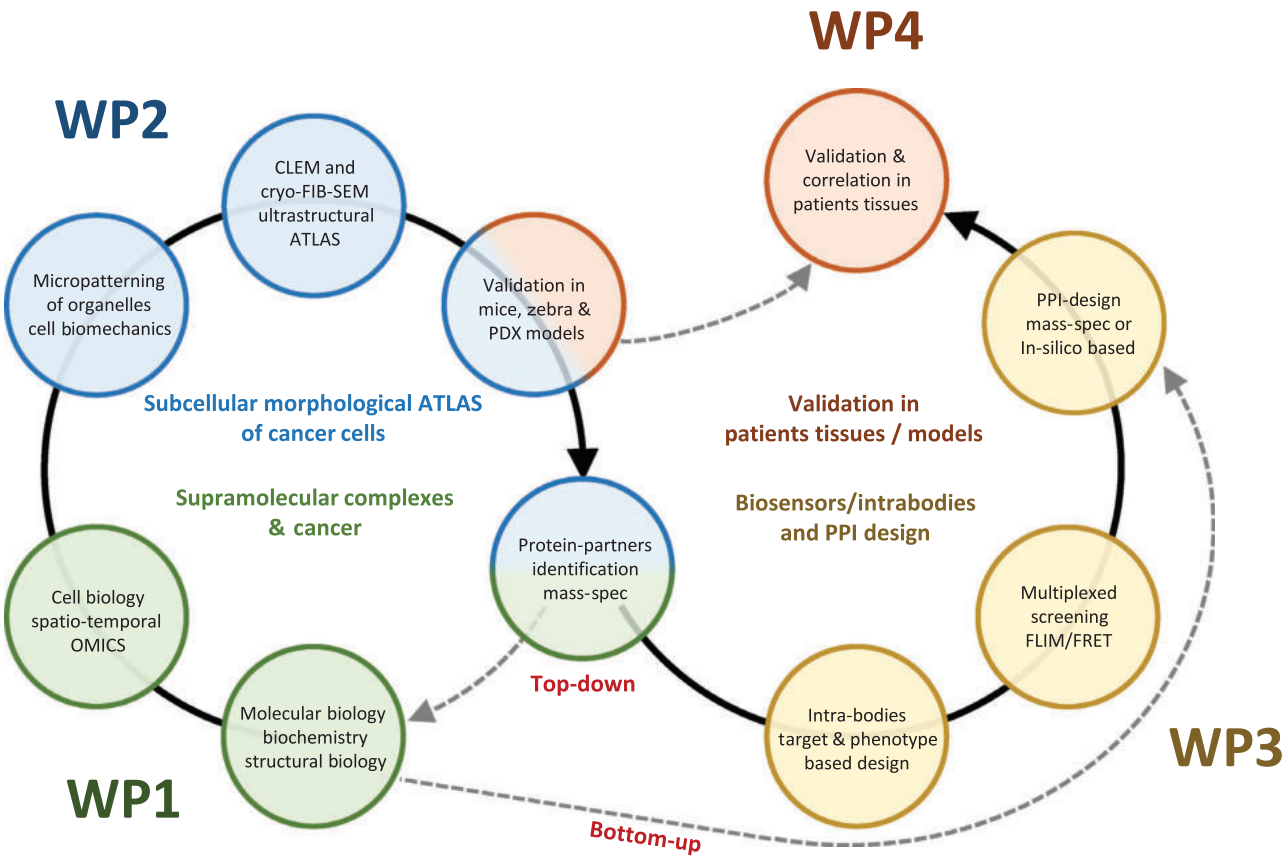
The NANOTUMOR consortium is establishing a new, transdisciplinary and collaborative community, structured around four work packages (WPs) with specific objectives (Figure 2). These WPs were designed and structured during initial steps of the construction of the consortium and thus represent integrated interests of the 13 selected partners, whose expertise are highly complementary (Figure 3). We aim at (WP1) isolating, characterising the protein composition and determining the structure of key molecular complexes involved in cancer onset and progression; at (WP2) quantifying alterations of intracellular organelles during the cancer cascade and identifying and characterising protein complexes that sustain oncogenic intracellular signature; at (WP3) developing biosensors, protein and chemical inhibitors to characterise *in cellulo* the molecular or cellular targets and modifying their properties by targeted inhibitors, and finally at (WP4) validating the targeted candidate protein complexes *in vivo* on patients. The acronyms used in the following paragraphs are defined in Table 1.

WP1: Molecular and structural study of keys protein complexes

While cancer was originally mostly explained by accumulation of genetic abnormalities over time, including mutations in oncogenes and tumour-suppressor genes, it is now well accepted that cancer is also driven by epigenetic changes (Jones & Baylin, 2002; Jones & Laird, 1999). Epigenetic modifications are implemented by multi-subunit molecular machines, which integrate multiple enzymatic, regulatory and signalling functions. These machines are frequently mutated in cancer patients, such as

Figure 2 | Structure and Logic of the Consortium's Research Axes

WP1 objective is to isolate, characterise the protein composition and determine the structure of key molecular complexes involved in cancer onset and progression. WP2 aim is to quantify alterations of intracellular organelles during the cancer cascade and identify and characterise protein complexes that sustain oncogenic intracellular signature. WP3 concentrate on the development of biosensors, protein and chemical inhibitors to characterise *in cellulo* the molecular or cellular targets and to modify their properties by targeted inhibitors. WP4 is designed to validate the targeted candidate protein complexes *in vivo* on patients' samples and PDX.



the three interlinked molecular complexes – namely H2A.Z and/or H3.3 containing complexes, the chromatin remodelling complex DAXX/ATRX, and the SWI/SNF complexes – that have been implicated in cancer onset, development and cancer drug resistance (Jiao et al., 2011; Kadoch & Crabtree, 2015; Schwartzentruber et al., 2012). A few challenges remain: studying the composition and interaction properties of these supramolecular complexes, determining their molecular architecture and describing their cellular activity throughout the oncogenic cascade in order to understand their mechanisms of action. Moreover, it is essential to study and understand how mutations identified in cancer patients perturb these properties. One potent strategy to achieve these goals, and which will be undertaken within this WP, relies on purification of these molecular complexes and structural analysis at atomic resolution that can be achieved by single particle cryo-EM. This approach will be complemented by state-of-the-art proximal interaction technologies (i.e., using TurboID) coupled with mass spectrometry to determine the peripheral protein interaction networks. In addition, spatio-temporal single-cell OMICS will be used to monitor the transcriptional status upon complex modifications. These will also be implemented into model organisms and/or organoid cultures to test the epigenetic perturbations at different stages of cancer

Figure 3 | Co-partners of the NANOTUMOR consortium

The network currently consists of 13 teams; selected team spreads over 10 different research units in France on seven geographical sites (Lille, Lyon, Marseille, Montpellier, Paris, Rennes and Strasbourg).

Jacky G. Goetz
Vincent Hyenne
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Metastasis
Tumour invasion
EVs biomechanics
in vivo CLEM
microfluidics

Patrick Schultz
IGBMC - Illkirch

Structural biology
Transcription complexes
Chromatin remodelers
Single particle cryo-EM
FIB-SEM microscopy

Ali Hamiche
IGBMC - Illkirch

Chromatin biology
Epigenetic regulations
Genomic approaches
Mouse genetics and ES cells
Pediatric glioblastoma

Kristine Schauer
Institut Curie – Paris

Cell micropatterning
Intracellular morphology
Bladder cancer
Late endosomes/lysosomes

Pierre Martineau
IRCM - Montpellier

Therapeutic antibodies
Protein and antibody engineering
Targeted therapies
Biotherapies
Colorectal cancer

Jean-Paul Borg
CRCM – Marseille

Cancer biology
Protein networks
in vivo studies
Breast cancer

Philippe Rondé
LBP – Illkirch

Tumour migration
Invasion and microenvironment
3D super-resolution microscopy
Melanoma

Izabela Sumara
IGBMC - Illkirch

Cell cycle
Ubiquitin signaling
High-throughput screening

Jan Bednar
Stefan Dimitrov
IAB - Marseille

Chromatin structure
Cryo-EM
Epigenetic regulation
Activity assays

Yves Colette
Xavier Morelli
CRCM – Marseille

Chem-informatics
High Throughput Screening
Chemical Libraries
Preclinical Models

Marc Tramier
Giulia Bertolin
M.-D. Gallibert
IGDR - Rennes

Fluorescence microscopy
FRET / FLIM
Mitochondria
Melanoma

Isabelle Fournier
PRISM - Lille

Mass spectrometry
Protein-protein interactions
Cross-linking MS
Molecular imaging

Marco A. Mendoza
Genoscope - Evry

Functional genomics
Organoids,
Spatial transcriptomics

Table 1 | Glossary

3D-STORM	Stochastic optical reconstruction microscopy
Cryo-EM	Single particle cryo-electron microscopy
(Cryo-) FIB-SEM	Focused ion beam milling combined with scanning electron microscopy
FRET/FLIM	Multiplex or classic Förster's resonance energy transfer of biosensors, measured by fluorescence lifetime imaging microscopy
Intravital CLEM	Correlative light and electron microscopy, in living organism
LOPIT	Localisation of organelle proteins by isotope tagging
OMICS	In this context, refers to proteomics and transciptomics
PDXs	Patient-derived xenografts
TurboID	Optimised biotin ligase for enzyme-catalysed proximity labelling (PL)

development or during resistance, notably in the context of paediatric glioblastoma which is at the heart of our efforts for WP1. From the study of these machineries, either involved or mutated in several cancer types, we expect to describe cancer-specific anomalies in their structure and activities. Moreover, our ambition is to identify epigenetic co-factors and other interactors that sustain the epigenetic modifications in cancer and the alterations in cell cycle regulation. Improved target identification and established atomic structures will be instrumental for *in silico* design of protein–protein interaction inhibitors in WP3 (Figure 2 – bottom-up).

WP2: Building an ultrastructural atlas of cancer cells

In addition to studying changes in cancer that occur in the nucleus, another ambition of this project is to establish an atlas of alterations of key intracellular organelles during the cancer cascade based on the models most used by the members of the consortium, namely breast cancer, bladder cancer and melanoma in WP2. The availability of cell lines, zebrafish, mouse, patient-derived xenograft (PDX) models and patient biopsies for breast cancer through the consortium will make it the convergent cancer model across WP2, WP3 and WP4 in a later stage. Because organelles carry out distinct cellular functions, this knowledge will provide a framework to stratify cancer cell deregulations. Our idea is to perform an unprecedented top-down strategy going from cancerogenic organelle alterations (including positioning) to higher order protein complexes and their networks, and finally to specific druggable targets of therapeutic relevance (Figure 2 – top-down). Our objective is to first establish higher order organelle alterations as morphological signatures of cancer progression using an interdisciplinary toolkit of organelle tracking under controlled cell culture conditions based on micropatterning and electron microscopy methodologies such as intravital correlative light and electron microscopy (Karreman et al., 2016) and FIB-SEM technologies (Spehner et al., 2020) to build an atlas of micro-to-nano scale deformations. Using preliminary results from co-partners of the consortium, targeted organelles will include late endosome/lysosomes in breast cancer, bladder cancer and melanoma cell lines. In later stages, the program aims at documenting additional organelles such as the nucleus, mitochondria, Golgi apparatus and centrosomes whose numbers, densities and morphologies are likely to be impacted and influential throughout cancer progression (Gensbittel et al., 2020). Identified alterations will guide us towards the molecular machineries/complexes that sustain higher order organelle alterations of cancer cells. Based on recently published work of the consortium (Ghoroghi et al., 2021), we have unanimously selected two GTPases of the Ral family (RalA and RalB) acting downstream of RAS, as proof of concept for the top-down efforts of WP2. More generally, potent approaches such as localisation of organelle proteins by isotope tagging (Geladaki et al., 2019; Mulvey et al., 2017) as well as proximal interaction technologies using TurboID coupled to mass spectrometry will allow the identification of such complexes (Branon et al., 2018). Complementary, high-end dynamic light microscopy techniques such as FRET/FLIM (Bertolin et al., 2016) and space and time super-resolved imaging using like 3D-STORM will allow live and resolutive tracking of these complexes and their interactions, and provide the technological backbone for the characterisation of complexes identified by this top-down strategy. While the consortium is centred around the two axes represented by WP1 (structural and molecular analyses of complexes involved in cancer) and WP2 (ultrastructural mapping efforts on different cancers), two additional high-risk high-gain translational WPs have been designed.

WP3: develop and screen biosensors, protein and chemical inhibitors

The identification of new therapeutic targets and specific inhibitors remains the key to develop new medicines (Swinney & Anthony, 2011). So far, all the methods used to reach these objectives employ a two-step process that either first identify a suitable target before designing or screening for chemical inhibitors, (WP3 bottom-up approach), or screen for inhibitors in a cellular system for a given phenotypic change and identify the drug target in a second stage (WP3 top-down approach). The main objective of the WP3 is to implement both approaches based on the results generated in WP1 and WP2. The high quality of the 3D structural characterisations of macromolecular complexes generated in WP1 will allow to implement bottom-up approaches to identify original protein–protein inhibitors (PPI) that target the

identified pathogenic protein–protein interactions. This will be initiated by a *in silico* docking and virtual screening of chemicals against these protein complexes protein–protein interface, and then evaluated *in vitro* by homogeneous time resolved fluorescence or ELISA. The identified hits will then be evaluated in a relevant cellular model (proliferation, invasion, migration, etc.) and allowed to identify compounds that have an inhibitory effect. Eventually, in all cases, the effect of the chemical PPI will be fully characterised in cell lines (patient-derived cells when available) and in animal models. Efforts in WP1 and WP2 will be concentrated on a very small number of molecular complexes and emphasis is given to the exploration of the molecular mechanisms using a large panel of complementary methods. In addition, we will implement a top-down complementary approach based on genetically encoded protein inhibitor screens (intrabodies) to identify original targets and inhibitors that revert the cancer phenotype previously identified (WP2). We will start from a semi-targeted approach to generate a collection of intrabodies that will be tested in cells to narrow the diversity down to a small number of efficient inhibitors (Mazuc et al., 2014). Such cellular screens will require the optimisation of intrabody libraries, high-throughput screening systems by FRET/FLIM, in-cell proteomics to identify and characterise the intrabody-induced disturbed protein networks before performing the PPI screening. The main advantage of this top-down approach is that the selection of the inhibitor intrabody facilitates the identification of the target. Indeed, the intrabody will serve as a traditional high affinity tool to perform immuno-precipitation, TurboID, chemical crosslinking and mass spectrometry analysis. In addition, the fact that the inhibitor is genetically encoded opens the way towards large-scale screening of cell populations using cell sorting and next-generation sequencing (Mazuc et al., 2014). Since the implemented strategy will directly look at oncogenic-specific events identified in WP2 this strategy will confer a high degree of selectivity to the identified inhibitors for subsequent therapeutic developments.

WP4: integrate and validate the targeted candidates *in vivo*

An important aim of our program is to quickly validate our findings in cancer patient primary tissues (both *in vitro* and *in vivo*) such as for example through the use of established PDX models in mouse or zebrafish. An exciting approach will be to define the spatial transcriptomics profile of primary tumours and metastatic evolution from animal model systems to link the behaviour of cancer cells – for example, adhesion or extravasation potential of circulating tumour cells – with their transcriptional program. We also have the ambition to validate whether the over- or under-expression of key proteins forming the complexes identified in previous WPs can be correlated with clinical features using human samples. Finally, it will be important to explore the therapeutic potency of drug inhibitors generated in WP3, alone or in combination with standard therapy, using established preclinical models. This WP will build on tissue microarrays and PDXs with defined molecular alterations and linked clinical records that are available within the consortium (Charafe-Jauffret et al., 2013).

Partners and organisation

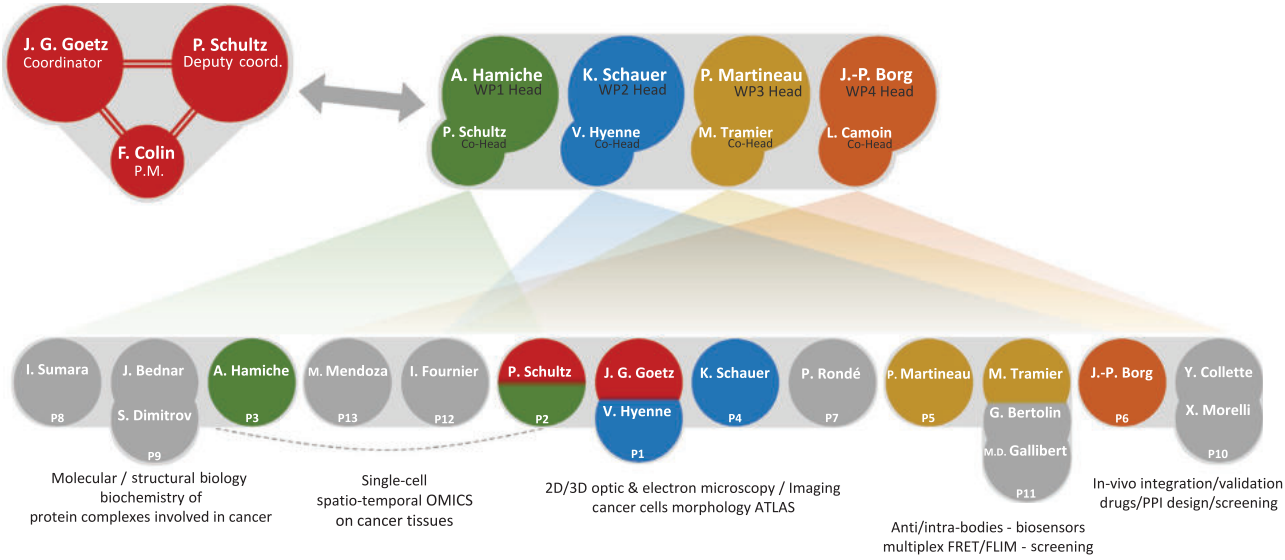
The NANOTUMOR consortium network currently consist of 13 teams. These are spread over 10 different research units in France on seven geographical sites and possess specific but very complementary expertise that shapes into a highly complementary and multi-disciplinary network (Figure 3). The consortium is open to evolution in its composition and to welcome international partners in a co-funding strategy. In addition to a global management led by two coordinators and a project manager, each WP is under the supervision of a head (with help/support provided by a co-head). Interactions between teams and WPs are optimised to take full advantage of the technical skills and solutions available in each group and at each site and also benefit from several long-term team interactions (Figure 4). This program is designed so that it allows the construction of a new French transdiciplinary community that – once we obtained sufficient preliminary results, increased our funding and attracted new international partners – interacts and exchanges with complementary and very large-scale initiatives such as the HTAN (Rozenblatt-Rosen et al., 2020).

Conclusion

The ambition of the NANOTUMOR Consortium is not only to establish stable links between the different teams, but also to create a synergy around

Figure 4 | Coordination of NANOTUMOR and Repartition in WPs

A global management is carried out by Jacky G. Goetz (U1109 – Strasbourg) and Patrick Schultz (IGBMC – Illkirch), with the help of Florent Colin, project manager (U1109 – Strasbourg). The four WPs are led respectively by Ali Hamiche (IGBMC – Illkirch), Kristine Schauer (Institut Curie – Paris), Pierre Martineau (IRCM – Montpellier) and Jean-Paul Borg (CRCM – Marseille). The involvement of each co-partner in the four axes of the program is highlighted.



this common project, and to obtain key scientific results to pave the way for further funding applications. Through a very active communication strategy and the organisation of NANOTUMOR events (such as meetings or conferences to be launched in 2021) focusing on the "Tumor Cell Atlas" concept, we wish to attract additional partners and support, locally or at the European and international level. Based on a strong technology background, the consortium should benefit from a leverage effect to create or strengthen existing infrastructures, to allow local researchers to initiate state-of-the-art projects with high-end technologies, and to develop new technologies for cancer research. Through the construction of a new community, where the search for complementary funding will be instrumental, we aim at studying cancer progression from a multi-disciplinary and multi-scale angle that will, hopefully, be beneficial to the patient.

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Conflict of interest statement

The authors have declared no conflict of interest.

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