MY WINNING ERC STG PROPOSAL

ERC National Information Day

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Before the ERC

Junior group leader, Lab of Cellular metabolism, IBT

- **Start in Oct 2020**
  - **New project**
    - insight from PhD and postdoc
    - very limited preliminary data
  - submitted Junior Star grant
  - feedback from the evaluators – mostly positive, not enough preliminary data, team leadership not yet proven

Before the ERC

Junior group leader, Lab of Cellular metabolism, IBT

- **2021**
  - working on preliminary data
  - team grew
  - ERC Stg proposal
    - support from a grant department of VIB – abstract, CV, brainstorming the idea with two VIB group leaders
    - support from the TC Prague – feedback on a presentation of the proposal idea
    - Mock interview before the 2nd round organized by TC Prague
  - awarded Junior Star and ERC STG

Structure of the proposal

- Challenge, knowledge gap & what is known
- Momentum, why me
- Hypothesis
- Impact

SECTION A: EXTENDED SYNOPSIS OF THE SCIENTIFIC PROPOSAL

CANCER METABOLISM v2.0 – ARE THE RELEVANT TARGETS IN CANCER METABOLISM MASKED BY TISSUE COMPLEXITY?

Altered metabolism is a hallmark of cancer. This represents a targetable vulnerability, but the treatments often suffer resistance and toxicity, possibly resulting from a metabolic complexity encountered *in vivo*[1-3] that is difficult to comprehend using traditional methods[4]. As an example, cancer cells were considered glycolytic when assessed in isolation[5] (referred to as the Warburg effect), yet *in vivo*, tumors may rely on the oxidative metabolism[3,6-8]. *In vivo*, the capacity of tumor cells to survive and proliferate depends on the availability of essential nutrients. These can either be (i) synthesized intrinsically or (ii) obtained from the environment through metabolic crosstalk. Therapies that block *de novo* synthesis of nutrients and building blocks, such as nucleotides[9], folate[10] and amino acids[11] might thus be blunted by compensatory salvage pathways. Targeting metabolic vulnerabilities of cancer cells, on the other hand, may lead to unwanted side-effects on other cell types.

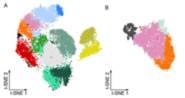

Figure 1. Heterogeneity of tumor endothelial cell (EC) assessed by scRNA-seq in **(A)** freshly isolated and **(B)** cultured tumor ECs isolated from human lung tumors. Each dot represents a single cell; different EC phenotypes are color-coded. Note that several EC phenotypes are lost in culture. Goveia, Rohlenova et al, 2020.

CHALLENGES: Cancer metabolism is a traditional field, but the majority of mechanistic insight was generated in culture using bulk techniques. But (i) the complexity of metabolic crosstalk between cell types or systemic pools of metabolites cannot be captured in culture models; (ii) gene expression (**Figure 1**) and metabolism are altered by culture conditions[4]; and (iii) bulk analyses mask the unique needs of cell types. → Are we lacking the resolution to embrace the full complexity of intercellular metabolic cross talk? Tissues consist of (metabolically) distinct phenotypes, **multi-omics analysis with single cell and topological resolution** is required to understand its complex biology and disentangle the intercellular crosstalk / metabolic trading patterns in healthy and tumor tissues *in vivo*. This project promises to **fill this gap in knowledge** and start a new era in cancer metabolism that, by embracing metabolite networks, can aid the development of new therapeutic strategies.

MOMENTUM: We now have a unique opportunity to uncover tissue complexity using integrated single cell multi-omics[12]. With the expertise & tools in hand, I am ready to take up this challenge. **PERSONAL EXPERIENCE:** Trained in cancer metabolism (PhD, anti-cancer compound passed a ph. I clinical trial[13]), during my postdoc I took the lead to set up the single cell expertise in the lab of P. Carmeliet, which resulted in studies that identified new targets in angiogenesis[1,14-17]. This involved scRNA-seq, big data handling, bioinformatics, target selection & validation, preparation of KOs. → I will focus on the **intercellular crosstalk of nucleotides in cancer,** where my objectives are to add a **new dimension** to our understanding of metabolic pathways.

RATIONALE

1. Rapidly growing (cancer) cells depend on availability of nucleotides, the building blocks of nucleic acids. As such, nucleotides are a target of antimetabolite therapy, in fact, the oldest widely used target in the history of cancer treatment.
2. Despite its long history, this treatment suffers high rates of resistance and toxicity for healthy tissue. → What are the reasons for toxicity and resistance? Are we missing important biological aspects of nucleotide metabolism *in vivo*? Can we find targets selective for tumor tissue and avoid toxicity?
3. Essentially, there are two ways a cell can gain nucleotides and its building blocks: (i) by *de novo* synthesis, (ii) by salvage pathways via recycling or from the environment. → Inhibition of *de novo* synthesis can be bypassed by nucleotides produced by surrounding cells or by distant organs.

HYPOTHESIS: I hypothesize that cancer cells differ from normal cells in how they utilize nucleic acid building blocks from internal and external sources and engage distinct mechanism to compensate for the DNS blockade. Therapeutic efficacy of nucleotide starvation is limited by salvage pathways via crosstalk of nucleotide building blocks with stromal cells, and alternative approach combining inhibition of DNS and cancer-specific salvage is required for successful elimination of cancer cells in their native environment.

IMPACT: The antimetabolites are administered systematically, exposing all cells in the organism to DNS inhibition. However, the adaptations to DNS inhibition might differ between cell types and in healthy vs. tumor tissue. Targeting these differences may open completely new horizons in fundamental tissue metabolism and identify new concepts for interventions that prevent resistance and toxicity.

Structure of the proposal

- State-of-the-art (tailored to the proposal)
- Research questions, key hypothesis, specific aims
- Work packages – time frame and personnel
  - Aim
  - Methodology
  - Outcome
  - Risk, gain & feasibility

Structure of the proposal

- State-of-the-art

BLOOD VESSELS & CANCER: Blood vessels are crucial component of tumor stroma and tumors stimulate their excessive growth, angiogenesis, to obtain oxygen and nutrients, but tumor vessels are structurally aberrant leading to uneven perfusion[3,27]. Metabolism of endothelial cells (ECs), the inner vessel lining, is rewired in tumors[1,28]. → ECs form the interface of circulation and tissues; their metabolic state might strongly influence the tissue environment. I will investigate the EC contribution to the nucleotide balance in tissues.

HETEROGENEOUS METABOLIC SIGNATURES IN TUMORS – NEED FOR SINGLE CELL MULTI-OMICS: A fundamental challenge in treating cancer is its inherent heterogeneity[29,30], and emerging adaptations to local gradients of metabolites and metabolic stress may lead to resistance[31]. The knowledge gained from (spatial) single cell omics needs to be transformed to precision medicine[32,33] upon integration with patient data. → Using a powerful combination of single cell RNA-sequencing (scRNA-seq) and *in situ* transcriptomics & metabolomics I will map the local metabolic networks upon DNS disruption.

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Lessons learned

- **Start early**
  - Get feedback from experts and non-experts
  - Give yourself time to digest the feedback
  - Fix all the recurring critique points

- **How to start**
  - Take courses in grant writing
  - Comment and edit grants from other people

One cannot get an ERC without applying for an ERC

Rohlenova lab

Funding