Metabolic Reprogramming of Exhausted T-Cells: Unlocking the Next Frontier of Checkpoint Blockade Refractoriness in Solid Tumors

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2025 Balzan Prize for Gene and Gene-Modified Cell Therapy

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Duration: 2026-2028

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1. Executive Summary

1.1 Project Overview and Strategic Intent

This research proposal delineates a comprehensive, three-year scientific program funded by the 2025 Balzan Prize. The project, titled *Metabolic Reprogramming of Exhausted T-Cells: Unlocking the Next Frontier of Checkpoint Blockade Refractoriness in Solid Tumors*, addresses one of the most critical challenges in modern oncology: the resistance of solid tumors to immune checkpoint blockade (ICB). While therapies targeting PD-1 and CTLA-4 have revolutionized cancer treatment, a significant subset of patients remains non-responsive due to the hostile, nutrient-deprived metabolic landscape of the tumor microenvironment (TME).

The core hypothesis of this proposal posits that T-cell exhaustion is not merely a transcriptional state driven by chronic antigen exposure but is fundamentally enforced by metabolic insufficiency. By decoding the metabolic checkpoints that parallel immune checkpoints, this project aims to restore effector function in exhausted T-cells (Tex) through targeted metabolic reprogramming.

This proposal is structured to support the advanced training and scientific contributions of two promising early-career scientists, **Angela Aznar Gomez**, **PhD**, and **Nour Shobaki**, **PhD**. In alignment with the Balzan Prize's objective to foster the next generation of researchers, this grant will provide three years of salary support, research funding, and a structured mentorship framework modeled after global best practices for high-impact scientific fellowships. The project leverages the prestige and financial backing of the Balzan Prize to bridge the gap between basic immunometabolism and translational therapeutic strategies, ensuring that the findings have a direct pathway to clinical application.

1.2 Strategic Alignment and Funding Utilization

The project utilizes half of the Balzan Prize to execute a high-risk, high-reward research agenda. The funding strategy is dual-pronged: providing immediate support for cutting-edge experimentation while simultaneously acting as a career accelerator for the designated fellows.

The allocation of funds reflects a strategic investment in human capital, recognizing that the advancement of science is inextricably linked to the development of the scientists themselves. By funding Dr. Gomez and Dr. Shobaki, the Balzan Prize is supporting two distinct but complementary research trajectories—experimental immunology and computational systems biology—that, when combined, offer a powerful approach to solving complex biological problems. This synergy is central to the project's design, which integrates wet-lab experimentation with advanced computational modeling to unravel the metabolic complexities of the TME.

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2. Scientific Background and Rationale

The Hypothesis: Metabolic Insufficiency Enforces Exhaustion

We hypothesize that metabolic insufficiency is not just a consequence of exhaustion but a causative enforcer. Specifically, we posit that chronic mitochondrial dysfunction—characterized by the accumulation of damaged mitochondria, loss of mitochondrial membrane potential, and defects in biogenesis (driven by PGC1α repression)—locks T-cells into an exhausted state that cannot be reversed by checkpoint blockade alone. The metabolic state of the T-cell dictates its epigenetic landscape, and without metabolic reprogramming, the epigenetic marks of exhaustion cannot be erased.

Therefore, "re-fueling" the T-cells through metabolic interventions may be the necessary partner to "re-releasing" the brakes via checkpoint blockade. This project will investigate specific metabolic regulators that can be targeted to reinvigorate mitochondrial health in TILs, thereby sensitizing resistant tumors to anti-PD-1 therapy. By addressing the metabolic underpinnings of exhaustion, we aim to develop a new class of immunotherapies that target the TME's metabolic landscape, offering hope to patients who are currently refractory to existing treatments.

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3. Personnel and Mentorship Environment

3.1 The Balzan Fellows

The success of this ambitious project relies on the expertise of two highly qualified postdoctoral scientists, Angela Aznar Gomez, PhD, and Nour Shobaki, PhD. Their complementary skill sets create a synergistic research environment capable of tackling the project's multidisciplinary challenges. The Balzan Prize funding provides a unique opportunity to support their development as independent investigators while advancing the field of cancer immunotherapy.

Angela Aznar Gomez, PhD: Lead Investigator (Experimental Immunology)

Dr. Gomez brings extensive experience in cellular immunology and *in vivo* tumor modeling. Her doctoral work focused on the regulation of cytotoxic T-lymphocyte (CTL) activation in chronic viral infections, providing her with a deep understanding of the exhaustion lineage. For this project, Dr. Gomez will lead the *in vivo* and *ex vivo* experimental arms.

- **Role:** Dr. Gomez will design and execute the murine tumor models, perform flow cytometric phenotyping of TILs, and lead the CRISPR-Cas9 screening efforts (Aim 2). She will also be responsible for the validation of metabolic targets using Seahorse extracellular flux analysis.
- **Career Trajectory:** Dr. Gomez is transitioning toward independence. This project will provide her with the leadership experience necessary to establish her own laboratory focused on therapeutic immunomodulation. The funding will allow her to develop a niche in metabolic regulation of T-cell function, positioning her as a leader in this emerging field.

Nour Shobaki, PhD: Lead Investigator (Computational & Systems Biology)

Dr. Shobaki is a computational biologist with specialized training in single-cell genomics and metabolic network modeling. Her background includes the development of algorithms to infer metabolic flux from transcriptomic data.

- **Role:** Dr. Shobaki will lead the high-dimensional data analysis components (Aim 1), including single-cell RNA sequencing (scRNA-seq) and SCENITH (Single Cell Energetic metabolism by profiling Translation Inhibition) analysis. She will construct the metabolic regulatory networks that guide the experimental targeting in Aim 2.
- **Career Trajectory:** Dr. Shobaki aims to bridge the gap between "dry" and "wet" labs. This project allows her to apply computational predictions to biological validation directly, a critical skill set for modern systems immunology. The funding will support her development of novel computational tools for immunometabolism, establishing her as an expert in the integration of multi-omics data.

3.2Mentorship and Career Development Plan

Drawing inspiration from world-class early career award programs, a structured mentorship plan has been designed for the Balzan Fellows. This plan ensures that the funding provides not just salary, but a trajectory for professional growth. The mentorship structure is designed to provide the fellows with the guidance, resources, and network they need to succeed in a competitive academic environment.

Phase 1: Mentored Research (Year 1)

- **Objective:** Establish technical mastery and generate foundational data.
- **Structure:** Weekly one-on-one meetings with the PI; bi-weekly lab meetings for data presentation. The fellows will also participate in institutional seminars and journal clubs to broaden their scientific horizons.
- **Milestone:** Submission of an abstract to a major international conference (e.g., AACR, SITC). The fellows will also begin drafting a review article on the intersection of metabolism and immunotherapy to establish their expertise in the field.

Phase 2: Project Leadership (Year 2)

- **Objective:** Develop independence in experimental design and junior mentoring.
- **Structure:** Dr. Gomez and Dr. Shobaki will begin mentoring a graduate student or technician. They will be responsible for the day-to-day management of their respective aims, including budget oversight and resource allocation.
- **Milestone:** Drafting of the primary research manuscript. The fellows will also present their work at a departmental retreat or symposium to gain experience in scientific communication.

Phase 3: Transition to Independence (Year 3)

- **Objective:** Preparation for faculty or senior scientist positions.
- **Structure:** The PI will provide dedicated guidance on grant writing, lab management, and networking. The Fellows will form a "Mentorship Committee" including external experts to provide diverse career advice (modeled on the PICI Bridge Fellow requirement [1]). This committee will meet biannually to review the fellows' progress and offer strategic advice on their career trajectories.
- **Milestone:** Submission of independent grant applications (e.g., NIH K99, ERC Starting Grant, or equivalent national awards). The fellows will also be encouraged to apply for faculty positions or senior research roles in industry, leveraging the network and reputation they have built during the fellowship.

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4. Implementation Plan and Timeline

This project is designed to be executed over 36 months, with clear milestones to ensure accountability and progress. The timeline allows for flexibility to adapt to experimental findings while maintaining a rigorous schedule for data generation and dissemination.

Year 1: Landscape Mapping and Tool Validation

- **Q1-Q2:**
  - Establish B16 and MC38 tumor cohorts (Dr. Gomez).
  - Optimize flow cytometry panels for metabolic markers (Glut1, CPT1a, MitoTracker).
  - Begin scRNA-seq library preparation (Dr. Gomez/Dr. Shobaki).
  - Establish collaborations with core facilities for sequencing and bioinformatics support.
- **Q3-Q4:**
  - Execute SCENITH profiling on TILs.
  - Computational analysis of single-cell data to identify metabolic clusters (Dr. Shobaki).
  - **Milestone:** Selection of Top 20 candidate genes for the CRISPR screen.
  - **Deliverable:** Year 1 Progress Report; Conference Abstract. The team will also hold a project review meeting to assess progress and adjust the experimental plan if necessary.

Year 2: Functional Screening and Target Identification

- **Q1-Q2:**
  - Cloning of the custom CRISPR library (Dr. Shobaki).
  - Generation of Cas9-P14 retroviral vectors.
  - Execution of the in vivo CRISPR screen in chronic LCMV and B16 models (Dr. Gomez).
  - Optimization of viral transduction protocols to ensure high efficiency and minimal toxicity.
- **Q3-Q4:**
  - Next-Generation Sequencing (NGS) of recovered T-cells to identify enriched/depleted sgRNAs.
  - Validation of top 3 hits using single-gene knockout vectors in competitive transfers.
  - **Milestone:** Identification of a validated "metabolic checkpoint" target.
  - **Deliverable:** Year 2 Progress Report; Draft of Manuscript 1 (Mapping). The team will begin drafting the manuscript for submission to a high-impact journal.

Year 3: Therapeutic Translation and Synthesis

- **Q1-Q2:**
  - Pre-clinical trials: Testing pharmacological inhibitors or gene-edited T-cells in combination with anti-PD-1 (Dr. Gomez).
  - Mechanistic studies: Assessing TME remodeling (macrophage polarization, cytokine milieu).
  - Assessment of off-target effects and potential toxicity of metabolic interventions.
- **Q3-Q4:**
  - Data synthesis and integration of "wet" and "dry" data.
  - Preparation of final manuscript (Functional Targeting).
  - Grant writing for future funding.
  - **Milestone:** Final Project Report; Submission of Manuscript 2 (Targeting). The team will also present the final results at a major international conference.