# Design and discovery of novel Granzyme B ligands and evaluation as diagnostics tools in CAR T-cell therapy assessment


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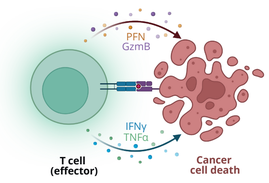
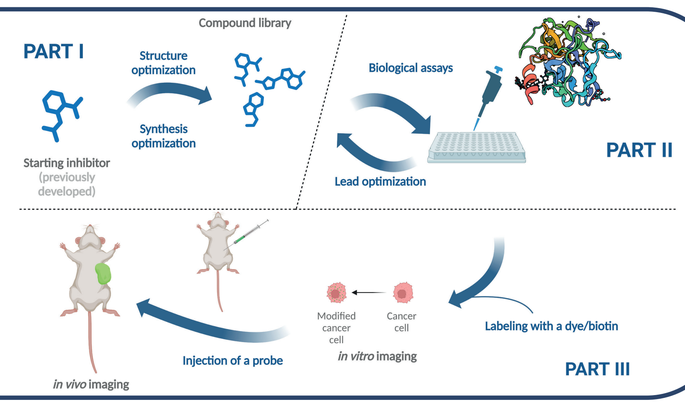
## INTRODUCTION

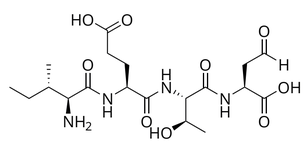
- **Granzyme B (Grnz B) is a serine protease** expressed in cytotoxic T-cells and natural killer (NK) cells that targets virus-infected cells and tumor cells.[1]
- Grnz B activates the **apoptotic pathway** by cleavage of caspases 8, 10, 3, 7.[1,2]
- Grnz B acts as a **signal factor in CAR T-cell immunotherapy.**
- Grnz B represents an intra-tumoral and extra-tumoral **target for molecular imaging** for **early cancer diagnostics and immunotherapy efficiency.**[3]



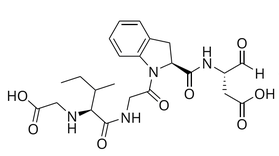




## AIMS AND OBJECTIVES

- Synthesis of a library of novel Grnz B inhibitors with a more drug-like profile to **overcome metabolic stability** and **cell-permeability** issues.
- *In vitro* biological evaluation of the compounds against **human Grnz B** and **caspases 3 and 10.**
- **Accumulating SAR data.**
- Selection of the **most suitable** (most potent & selective) **inhibitor** to be converted into a **probe** (attachment of a linker with a tag).



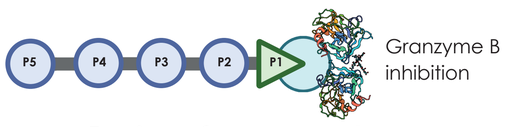
**IEDT** inhibitor[3]
**$K_I$ = 80 nM**



**VTI-1002** inhibitor[3]
**$K_I$ = 4 nM**

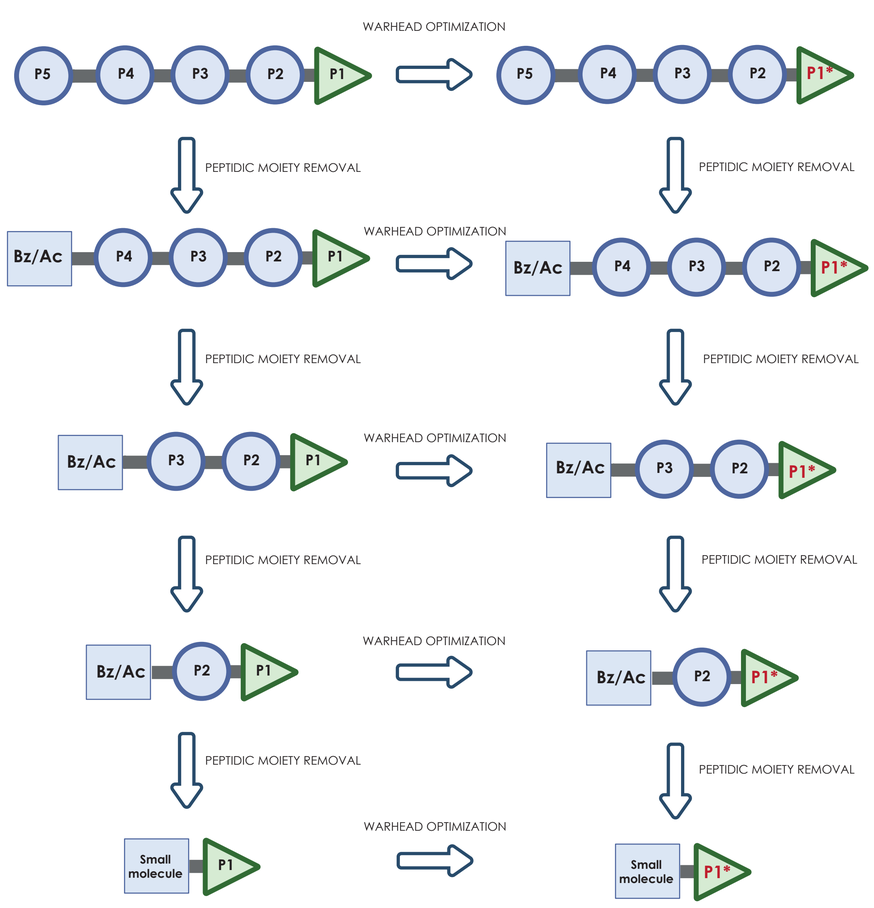
## METHOD AND RESULTS

### 1. COMPOUNDS LIBRARY SYNTHESIS




**Design of compounds**

**1st series:** **2nd series:**




- **Fully optimized** synthetic method for **2nd series**.
- **Almost optimized (90%)** synthesis for **1st series**.

**LEAD OPTIMIZATION**

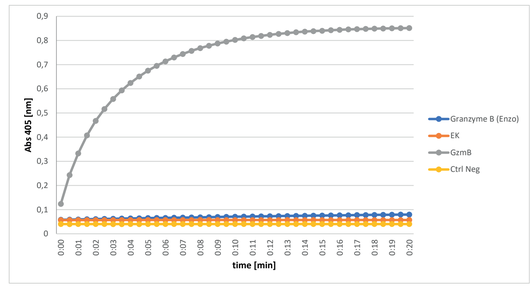
### 2. BIOLOGICAL ASSESSMENT

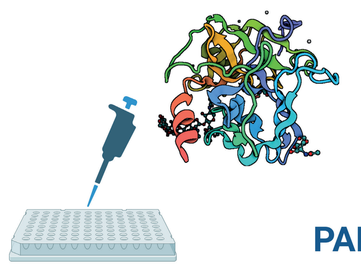
**Granzyme B expression** (performed by Dr. A. Fernandes)

**Source**: sf9 cells

**Activity tests**

*Substrate:* Ac-IEPD-p-NA.
c = 200 uL.
*Solution:* 50 mM Tris (pH 7.4), 100 mM NaCl, 10% glycerol.



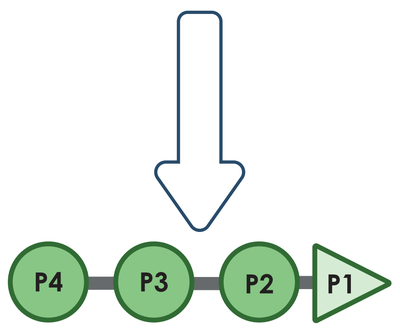



**PART II**

*In vitro* assessments:

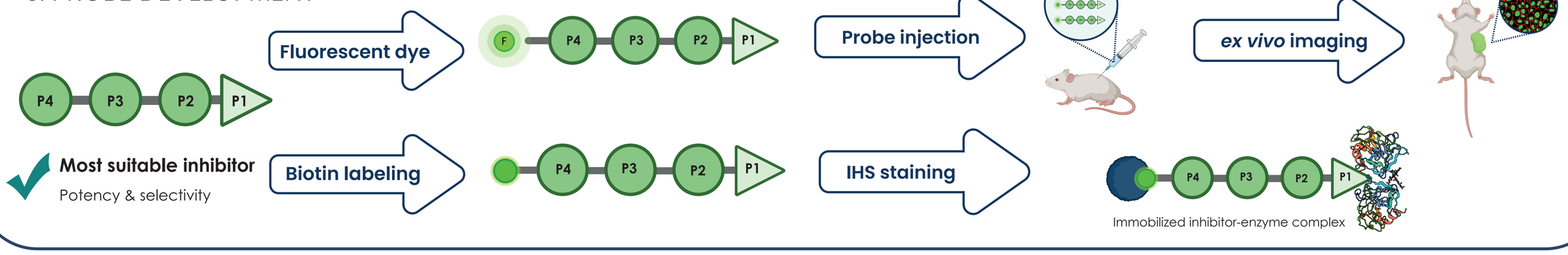
**Enzymes:**
- Human Grnz B.
- Mice Grnz B.
- Caspases 3, 8, 10.

**Tumor cells:**
- MC38.
- CT26.




**Most suitable inhibitor**
Potency & selectivity

### 3. PROBE DEVELOPMENT




## REFERENCES


1. *Angew. Chem. Int. Ed*. **2021**. 60 (11), 5699–5703.
2. *J. Biol. Chem*. **2020**. 295 (28), 9567–9582.
3. *Nature communications*. **2021**. 12(1), 1-14.
4. *Experimental & molecular medicine*. **2018**. 50(5), 1-11.



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