Deciphering the LILRB4 Immunosuppressive Landscape in Colorectal Tumors for De Novo Therapeutic Intervention

GPU Day
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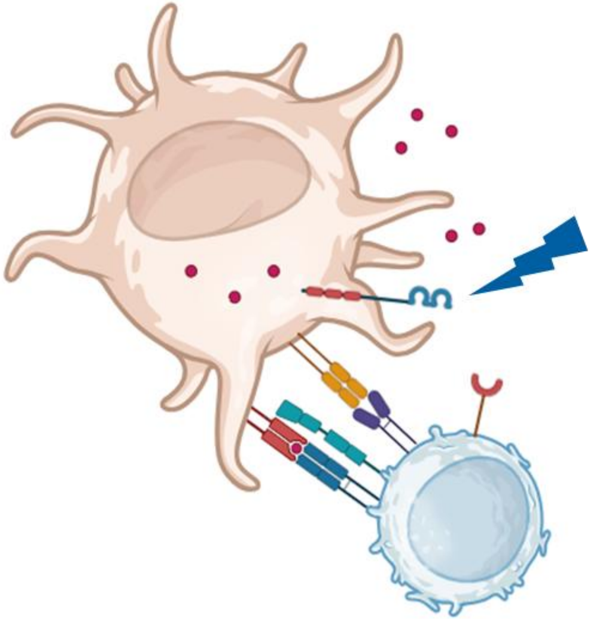
Péter Hunyadi
HUN-REN TKI
PhD Student @ Pázmány ITK
Biophysics & Radiation Biology, SE

LILRB receptors - immune checkpoint molecules

- Cancer is highly adaptive: avoids immune response
- Immunosuppression > immune checkpoint (ICs) molecules
- Ligands (expressed by tumor cells) bind ICs to suppress the immune cells



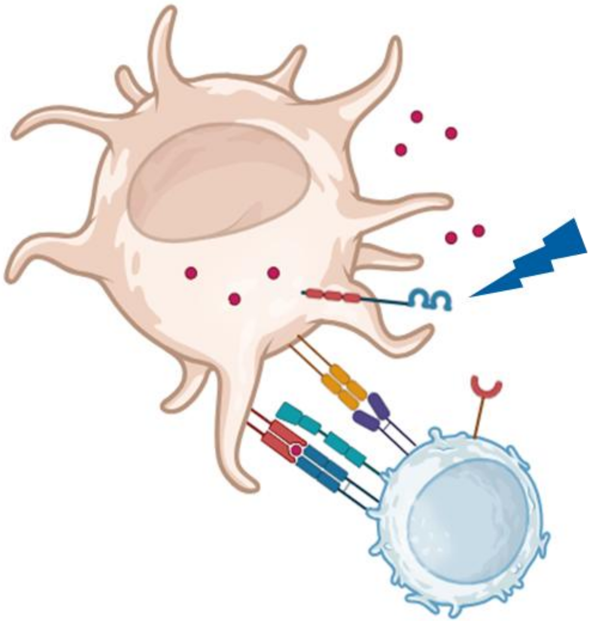
- LILRB receptor family are immune suppressor receptors
- **LILRB4:** antibody therapy on clinical trial

  > study in colorectal cancer (CRC) context

Study plan

- Inhibit LILRB4 function!

  In silico design of a **LILRB4** binding nanobody
- Test its ability *in vitro*



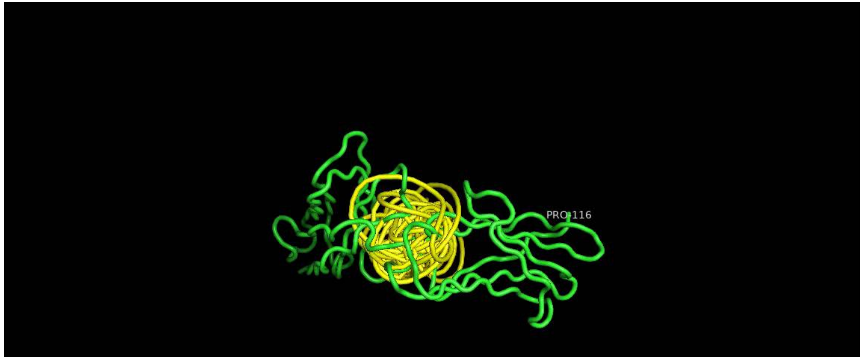
Challenges

1. Design a strongly binding **nanobody**

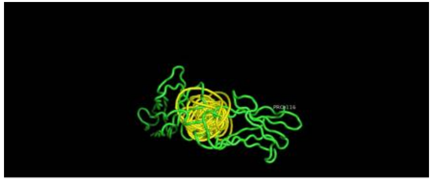
Nanobody design

Phase 1:
Backbone design

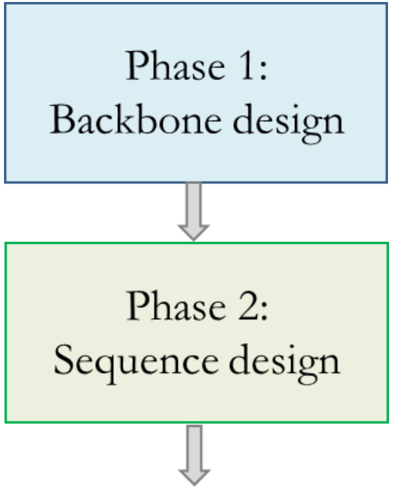
RFDiffusion
Start from constrained backbone
Diffusion introduces small changes



Nanobody design



Rfdiffusion/ RFantibody



Phase 1:
Backbone design

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Start from constrained backbone
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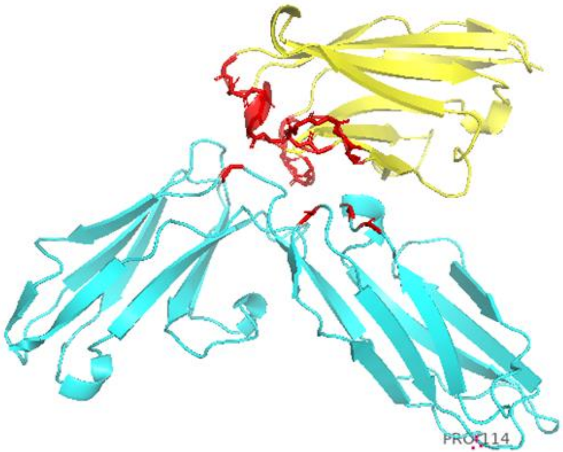
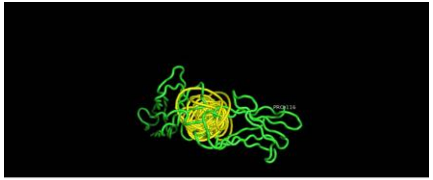
Phase 2:
Sequence design

Adds sequence to the structure
We get a lot of sequences this way



ProteinMPNN

Nanobody design



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Phase 3:
Filtering

AlphaFold3 predicts complexes well
Filter out incorrectly folding sequences

AF3

Conclusion

1. A set of nanobodies were designed *in silico* and their binding ability to LILRB4 is being validated in the lab right now.

Study plan

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  In silico design of a **LILRB4** binding nanobody
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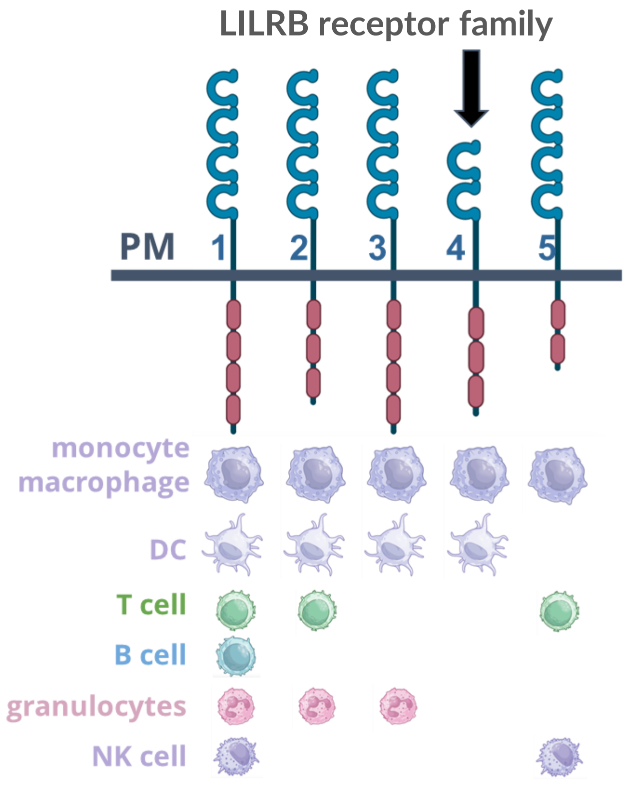
Challenges

1. Design a strongly binding **nanobody**
2. Identify LILRB4 expressing cells in CRC



single-cell RNA sequencing data

- RNA amount > good proxy for protein levels!
- Single-cell resolution
- Widely used (eg. monitoring of disease progression)

- Dataset used here:
  62 samples
  370.000 cells from colorectal tumors and adjacent normal tissues
  Pelka *et al.* Spatially organized multicellular immune hubs in human colorectal cancer. Cell. 2021

Cell type annotation with LLM

- Based on transcription difficult to guess cell type
- Precise annotation of single cells

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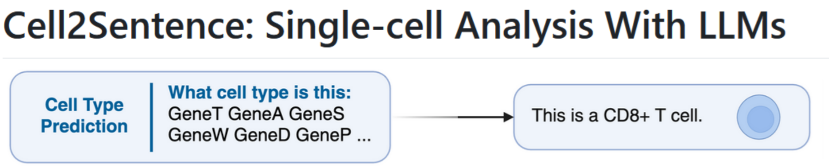
A new LLM based tool: Cell2Sentence (Google DeepMind/Yale 2024-25)

- 2B param. version - runs locally
- Input: “sentence” of most expressed gene names in descending order
- Good performance (88% accuracy) of cell type prediction, but at the cost of the ability to argue about the output!



Cell2Sentence: Single-cell Analysis With LLMs

Cell Type Prediction

What cell type is this:
GeneT GeneA GeneS
GeneW GeneD GeneP ...

This is a CD8+ T cell.

https://github.com/vandijklab/cell2sentence

Comparison with general LLM

Comparing with **Deepseek-v4-flash** general model

Comparison scheme:

1. Same prompts to analyse the same 1000 cells
2. LLM compares the two answers - rates correspondence 1-5
3. The scores are mined from the answers and summarized

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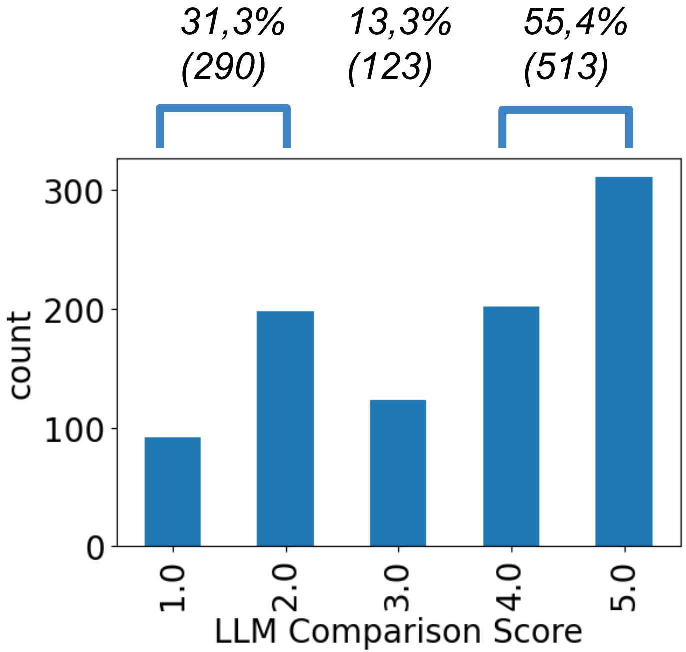
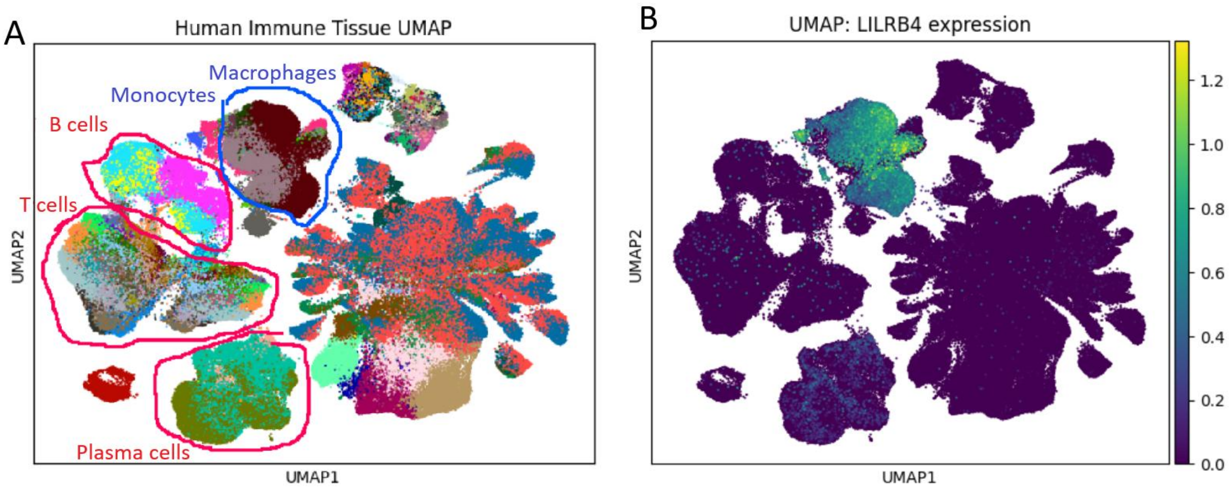
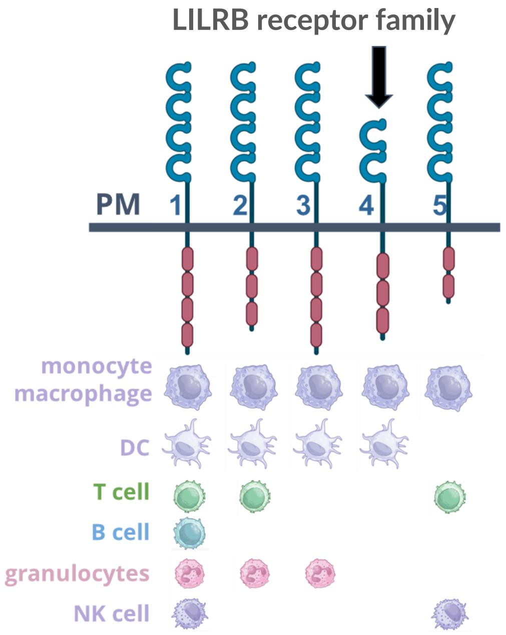
Results of the experiment

- ~40% difference suggests that **Deepseek is less accurate**
- Tradeoff is that **Deepseek argues about its output**, while **C2S does not have natural language answer capabilities** right now



LILRB4 is expressed by myeloid immune cells





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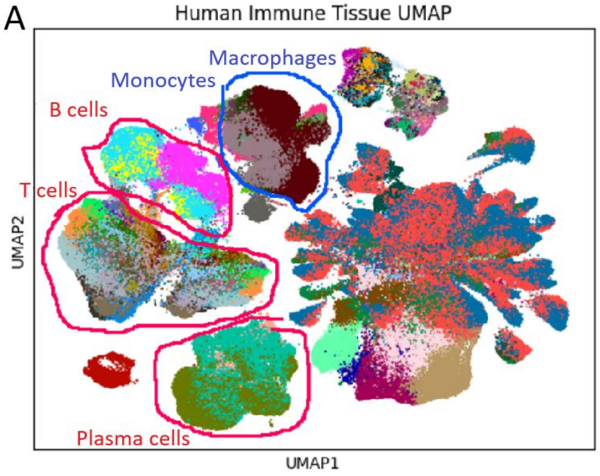

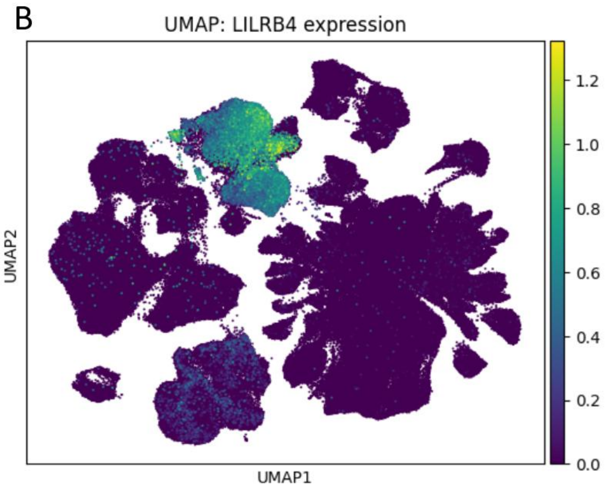

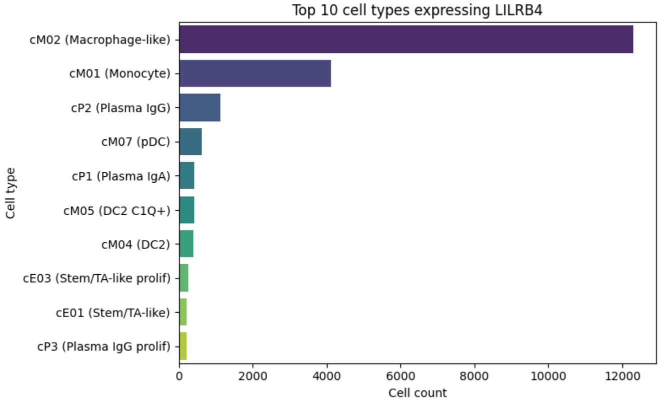

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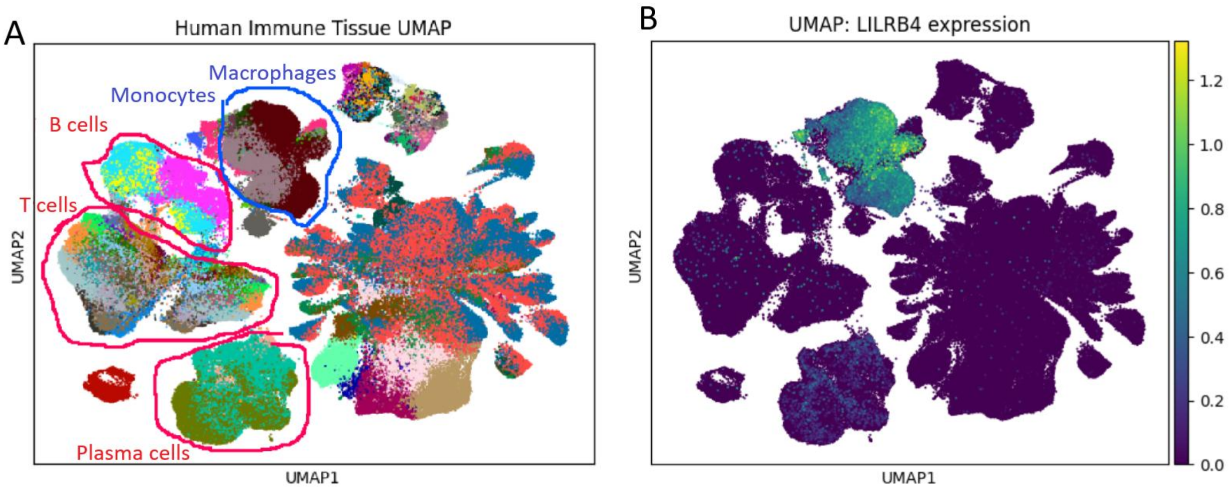
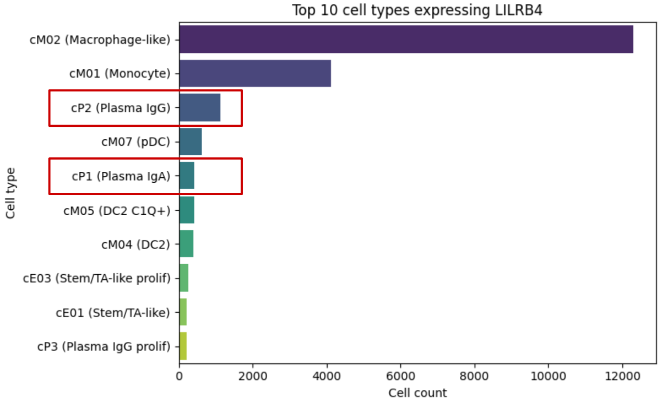


Interestingly we also found lymphoid immune cell expression!



Conclusion

1. A set of nanobodies were designed *in silico* and their binding ability to LILRB4 is being validated in the lab right now.

2. Comparing language model performance on biological tasks is an understudied, but important question.

- We argue for a trade-off between using a specialized and a general model

Thank you for your attention!

Contributors:

<u>Zoltán Wiener, Dávid Kis</u> and colleagues
Institute of Genetics, Cell- and Immunobiology
Semmelweis University, Budapest

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HUN-REN Wigner GPU-lab
KIFÜ HPC KK

Department of Biophysics and Radiation Biology
Semmelweis University, Budapest

Hegedűs Tamás
Tordai Hedvig
Kozák Eszter

Padányi Rita
Lór Kriszti
Gereben Orsolya

Berta Blanka
Lana Khamisi
Karen Torres