

# From encoded combinatorial libraries to clinical-stage therapeutics

**Dario Neri**

CEO and CSO of Philogen ([www.philogen.com](http://www.philogen.com))

Emeritus Professor of ETH Zürich

**ETH**

Eidgenössische Technische Hochschule Zürich  
Swiss Federal Institute of Technology Zurich

**Philogen**  
innovating targeting

# I have had good teachers

## 1982-1987

- Laurea in Chimica at the Scuola Normale Superiore (focus on Organic Chemistry)



## 1987-1992

- Ph.D. in Chemistry from the ETH Zürich working with Prof. Dr. Kurt Wüthrich (Nobel Prize Chemistry 2002)



## 1992-1996

- Post-doc at the LMB, MRC Centre in Cambridge working with Sir Gregory Winter (Nobel Prize Chemistry 2018)



# I have had good students

## 1996-now

- Professor at the Swiss Federal Institute of Technology (ETH Zürich)



## 2016-2020

- ERC Advanced Grant (a prestigious grant)



## 1996 and following years

- Co-Founder of Philogen (1996)
- Some of my students have started successful companies (e.g., Bicycle Therapeutics, Covagen, Allcyte)



# The Philogen group

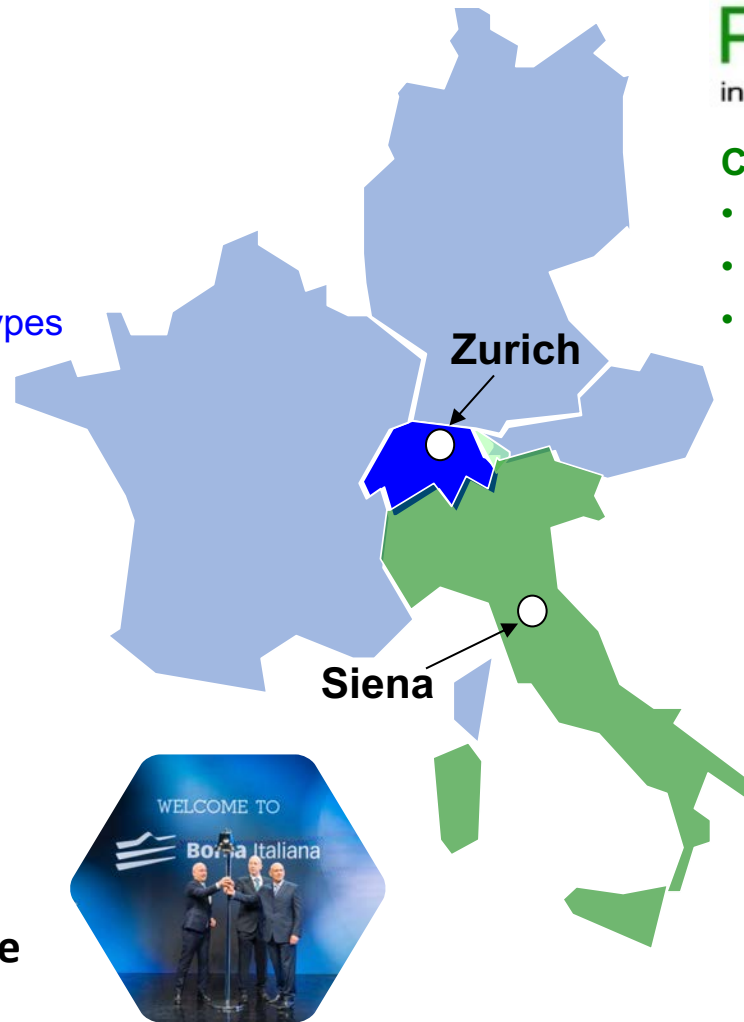
**Philochem**  
innovating chemistry

## Discovery Center

- Antibody technology
- DNA-Encoded Chemical Libraries
- Preclinical investigation of new prototypes



Listed on the Italian Stock Exchange



**Philogen**  
innovating targeting

## Clinical trial management and GMP-production

- Six-armed antibody products in clinical development
- Two fully-owned products in Phase III clinical trials
- In house GMP production facility



Collaborations with large pharmaceutical companies



abbvie

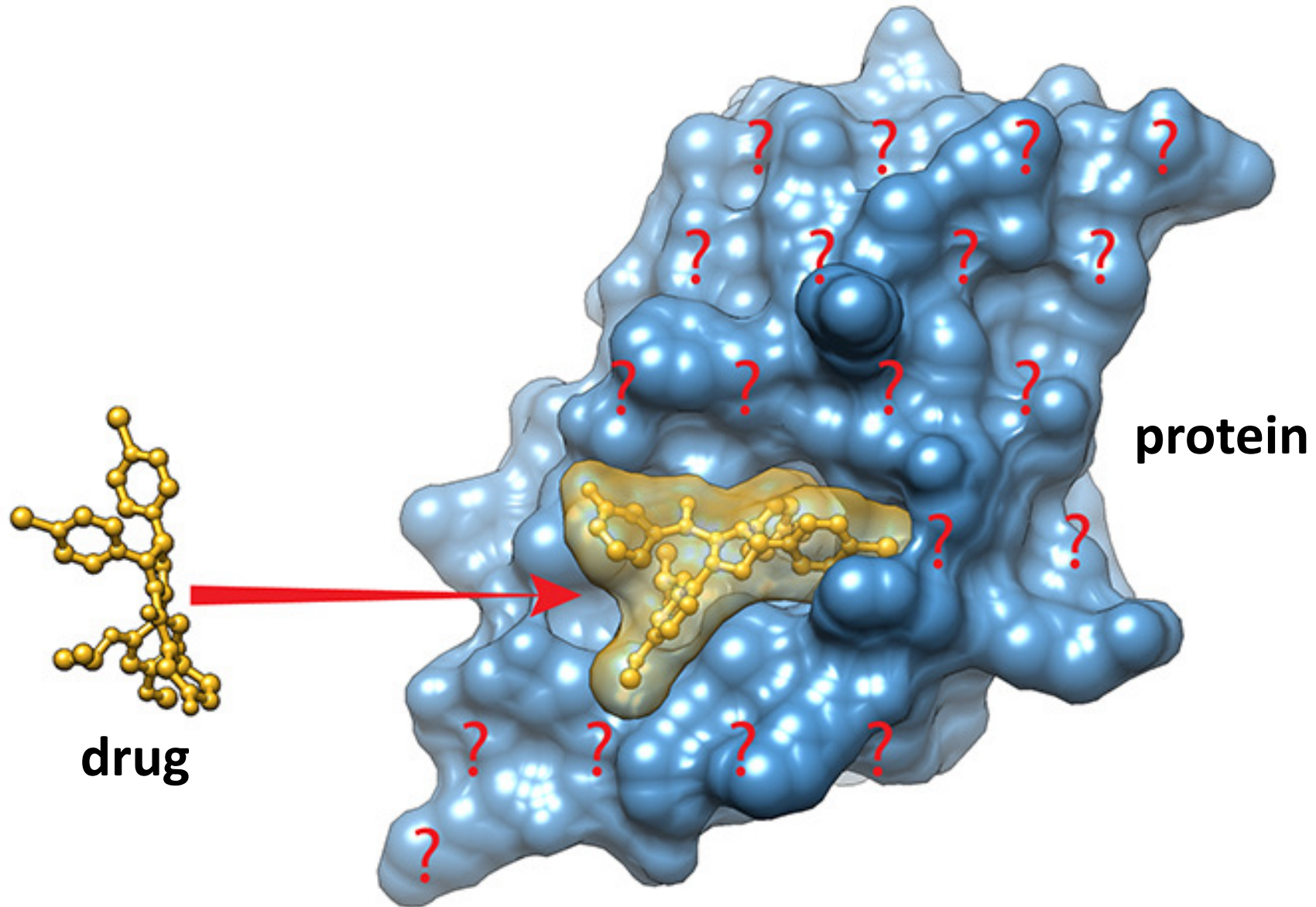
Johnson & Johnson



NOVARTIS

Bristol Myers Squibb™

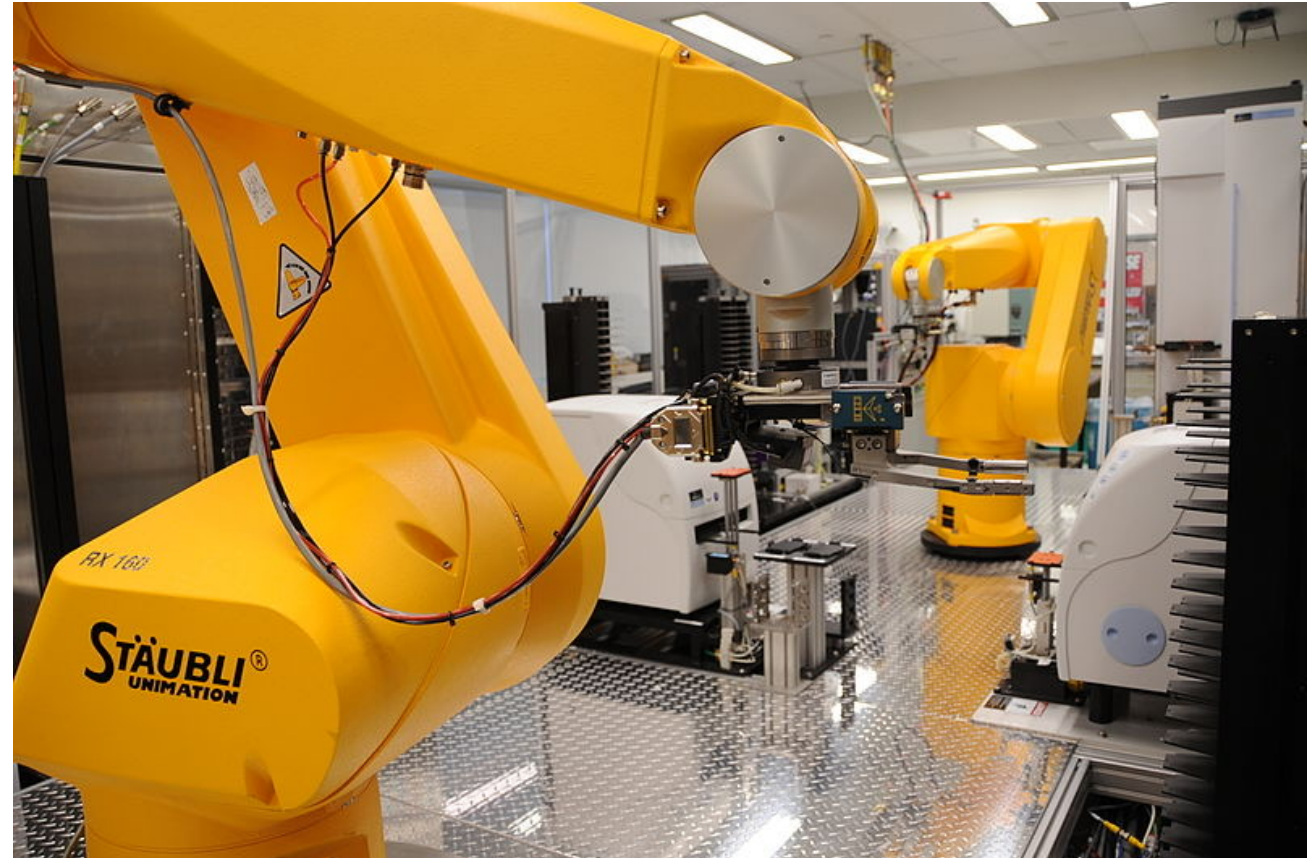
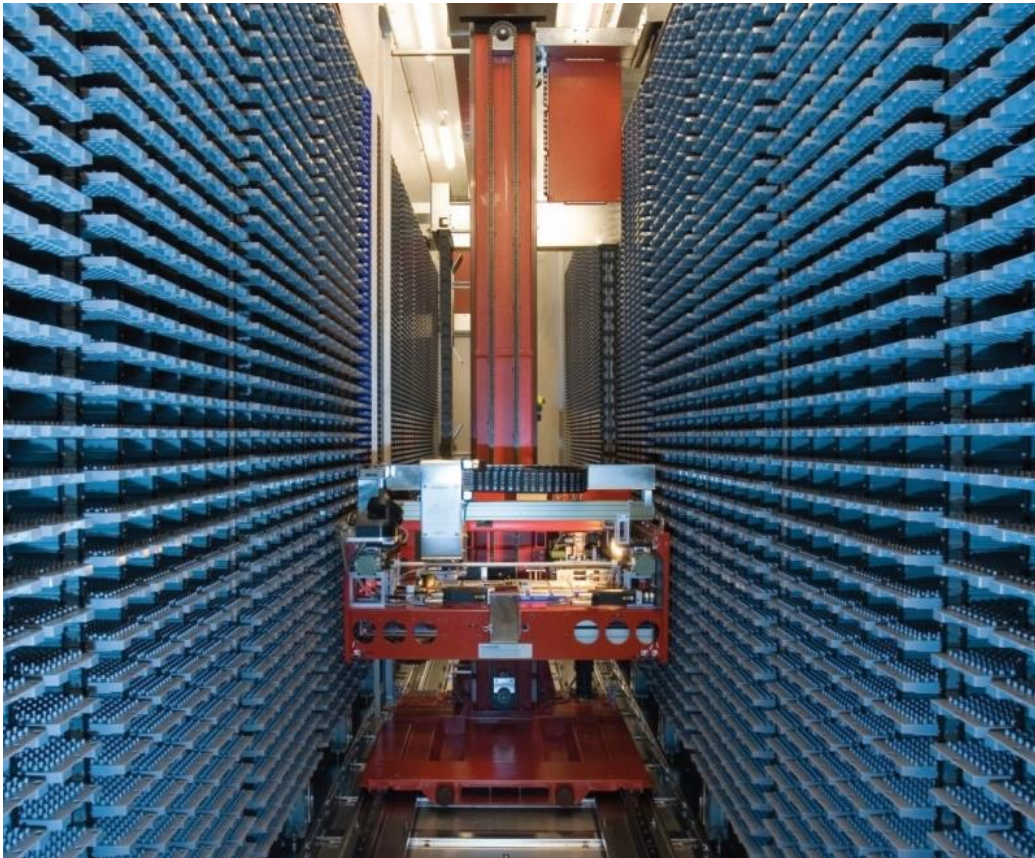
Virtually all **drugs** are molecules (big or small) which **bind** to a protein



# The inefficient way of discovering drugs (“one-by-one”)

The conventional (and inefficient) way of discovering drugs is to test molecules “one-by-one”

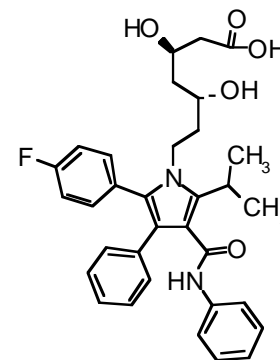
Even the largest companies cannot afford to screen more than one million compounds



# Therapeutic proteins vs. small organic drugs



Humira™ global net  
sales \$ 19.9 billion in 2018

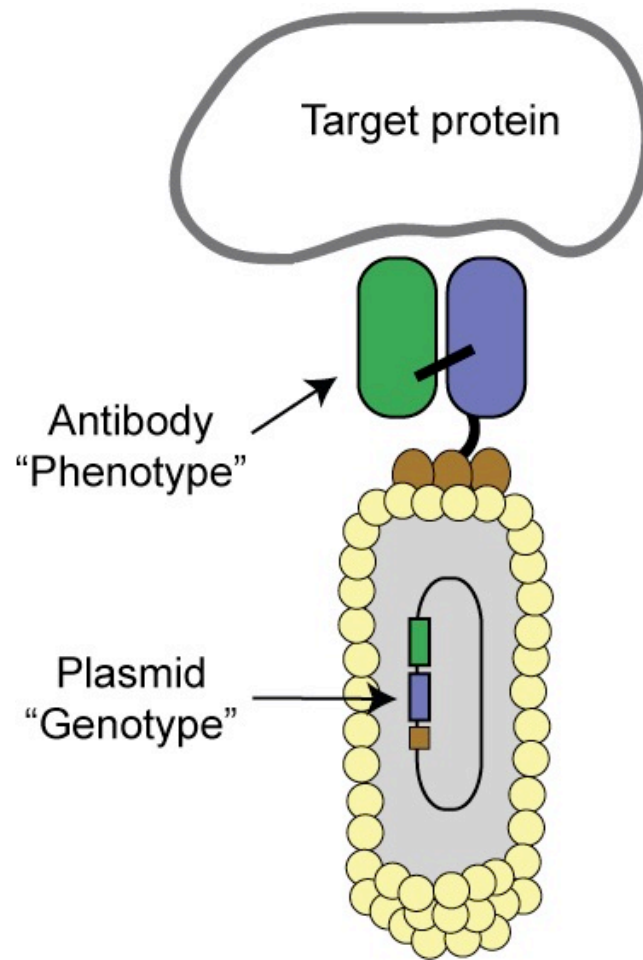


Atorvastatin™ global net  
sales \$13.6 billion in 2006

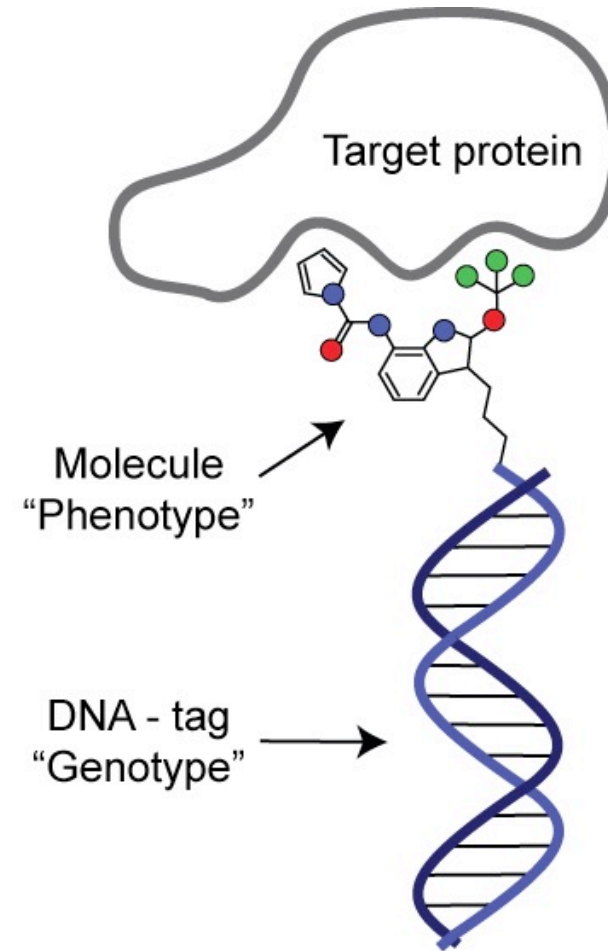
- 
- ~150'000 Da (large)
  - Cannot permeate cells
  - Long circulatory half-life
  - Must be injected
  - Highly specific
- ~ 500 Da (small)
  - Cell penetration possible
  - Typically shorter half-life in blood
  - Often orally available
  - May be less specific

# Encoded library technologies

Encoded combinatorial library technologies facilitate the discovery of antibodies and of small ligands



**Antibody Phage Display Library**

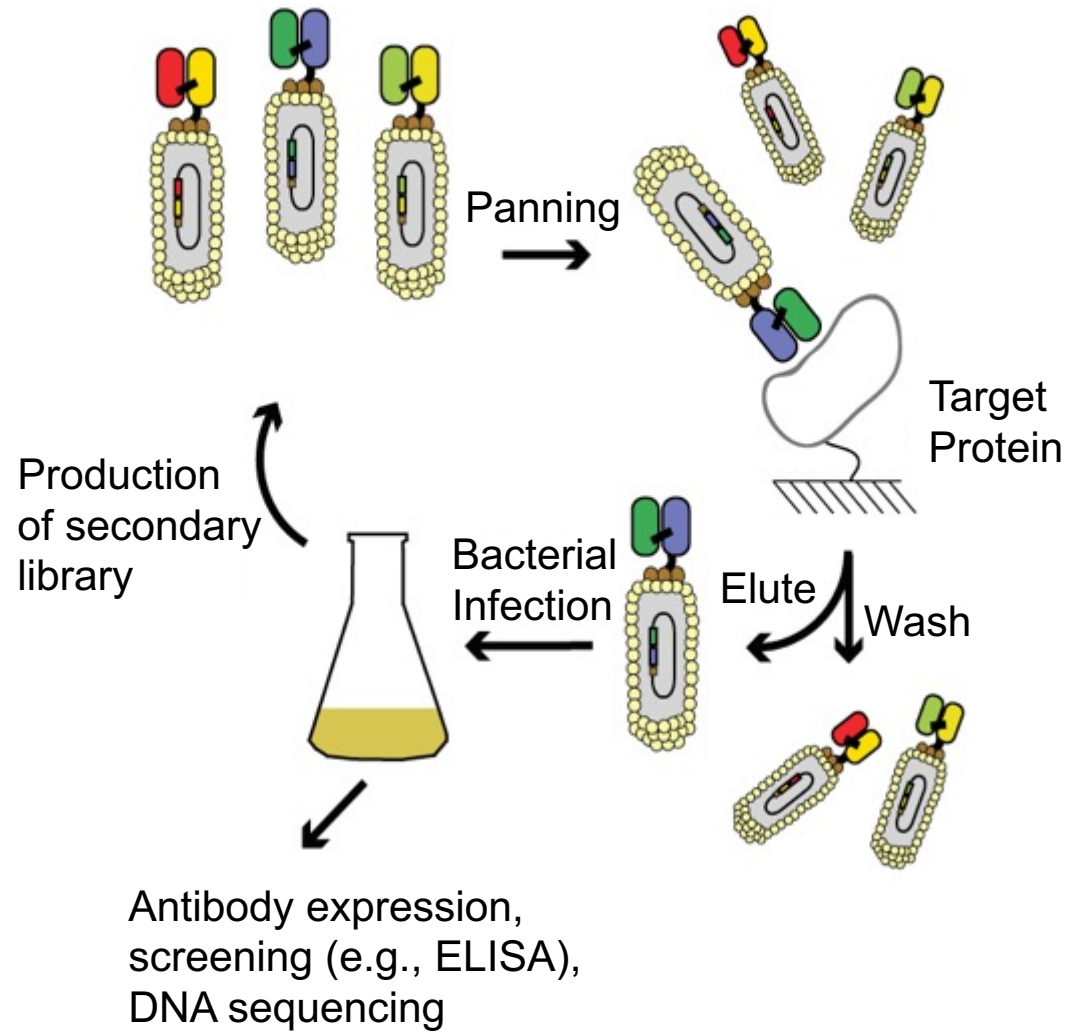


**DNA-Encoded Chemical Library**

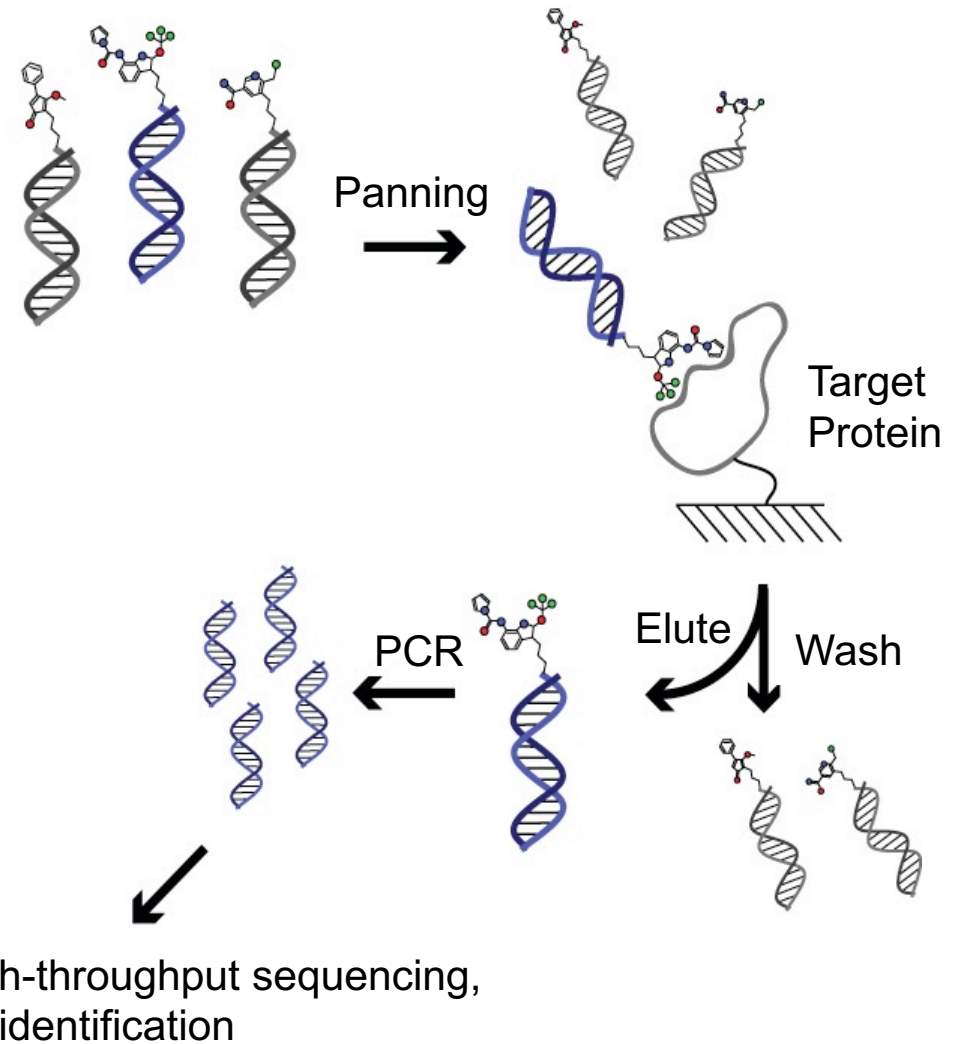


# Encoded library selections

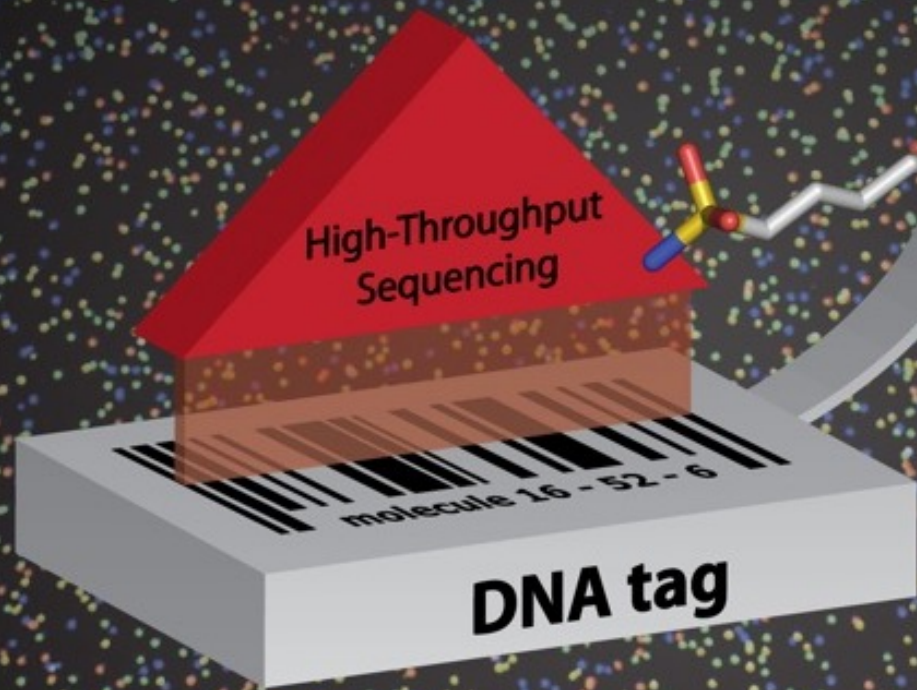
Antibody phage-display library  
(more than 100 billion different antibodies)



DNA-encoded chemical library  
(more than 1 billion molecules)



# DNA as “the” barcode



# A history of DEL technology

## Split & Pool on Beads

Richard Lerner & Sydney Brenner



Scripps Research

*Proc Natl Acad Sci USA*,  
1992, 89, 5381

1992

## First proposal

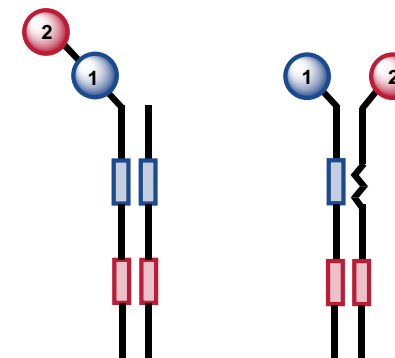
*of DNA-Encoded Chemical Libraries (e.g., peptides)*

## DNA-Recorded Synthesis

Dario Neri's lab

Philochem  
innovating chemistry

ETH zürich

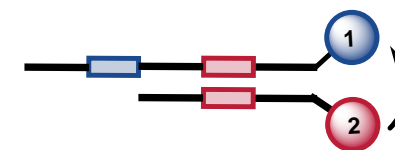


*Nat Biotechnol*,  
2004, 22, 568

*Proc Natl Acad Sci USA*,  
2008, 105, 17670

2004

HARVARD  
UNIVERSITY



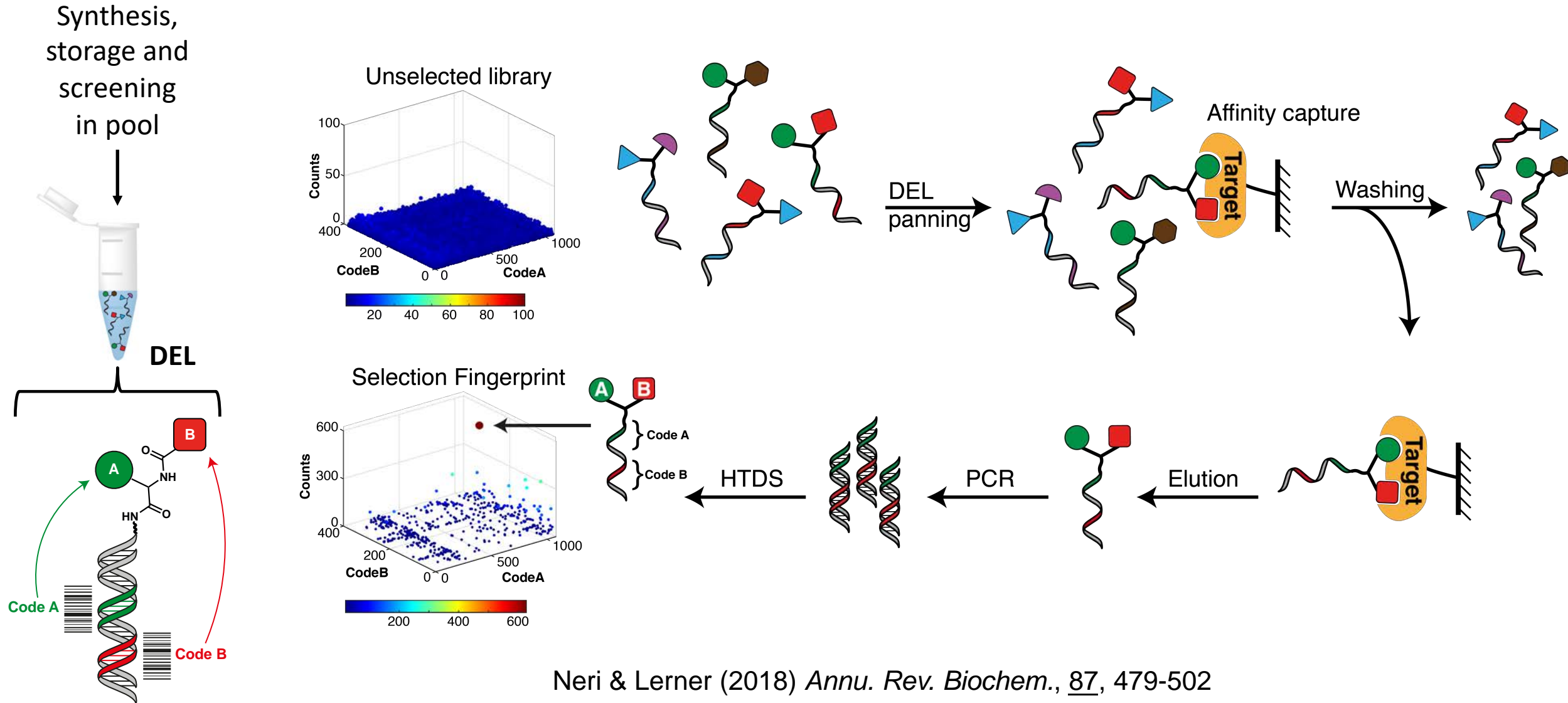
*Science*,  
2004, 305, 1601

David Liu's Lab

## DNA-Templated Synthesis

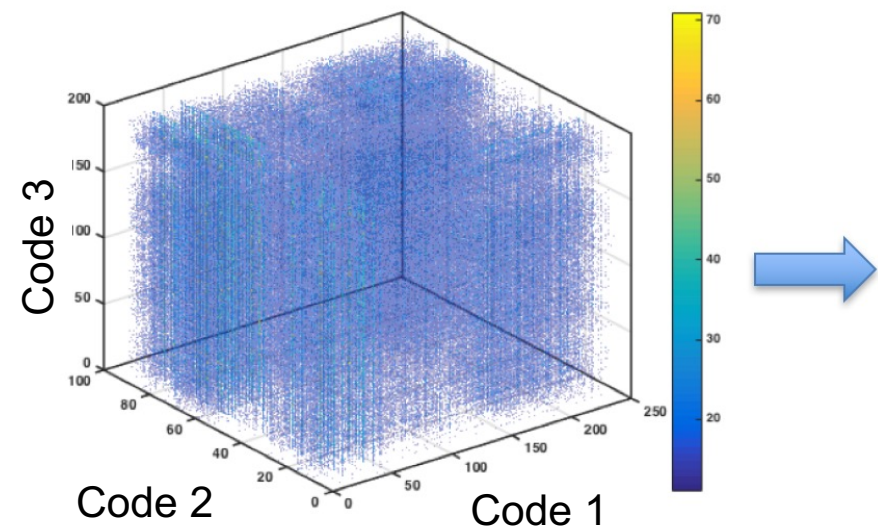
# Encoded library selections

Using combinatorial technologies, we can build and screen DEL libraries containing billions of different compounds

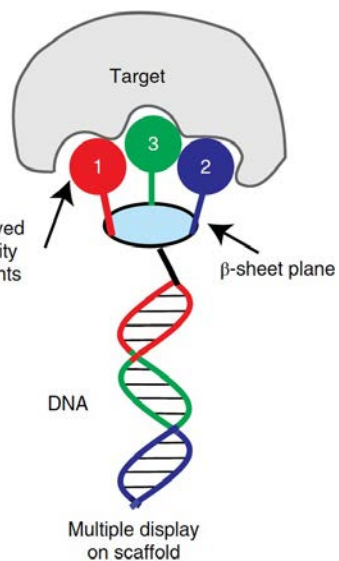


# DNA-encoded chemical library selections

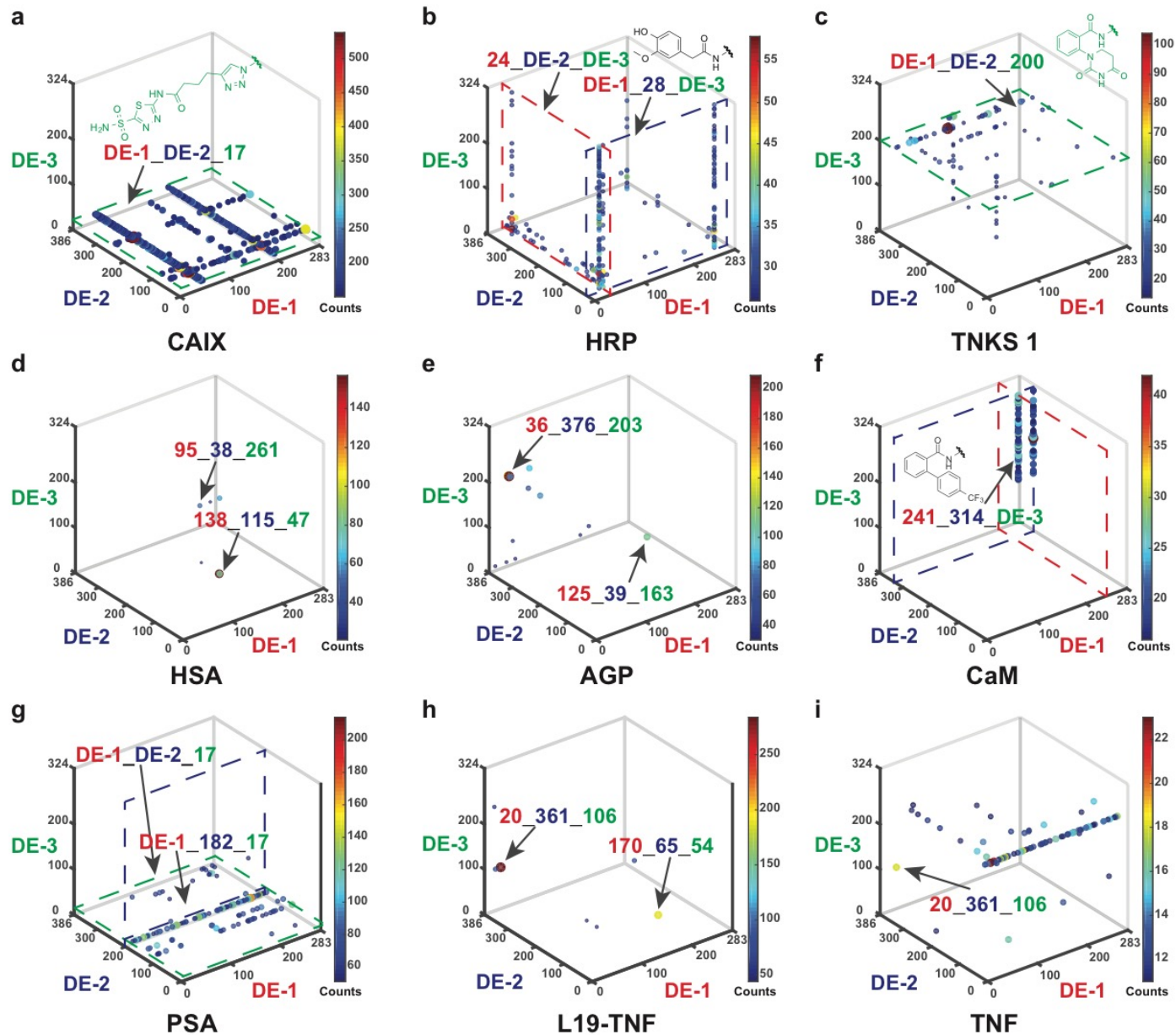
DELs may facilitate ligand discovery



Library before selections



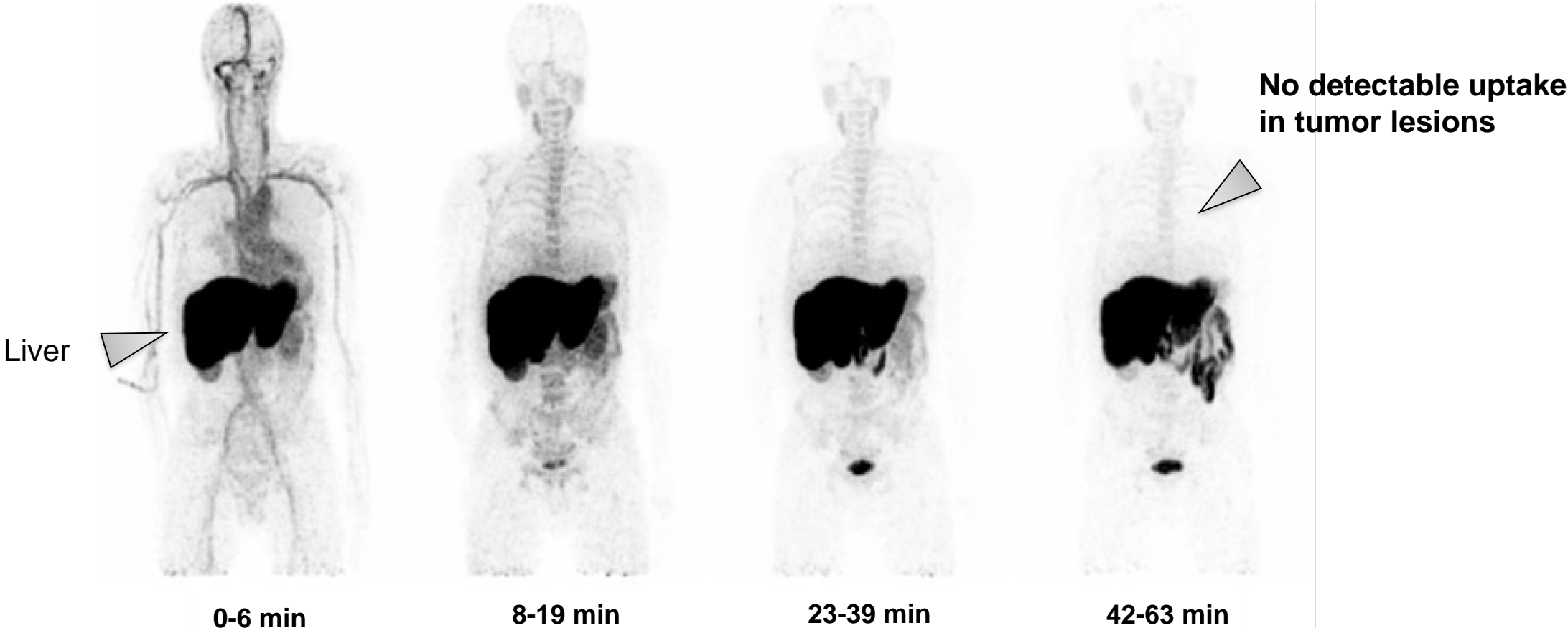
Li et al. (2018)  
*Nature Chemistry*, 10, 441



**The problem to be solved**

# Limitations of conventional chemotherapy

Conventional chemotherapy agents do not preferentially localize to solid tumors



PET imaging and biodistribution analysis of a patient with metastatic malignant mesothelioma, imaged with <sup>11</sup>C-docetaxel. Tumor lesions (in the pleura) **are not visible**.

van der Veldt *et al. Eur. J. Nucl. Med. Mol. Imaging* **2010**, 37, 1950; van der Veldt *et al. Clin. Cancer Res.* **2013**, 19, 4163

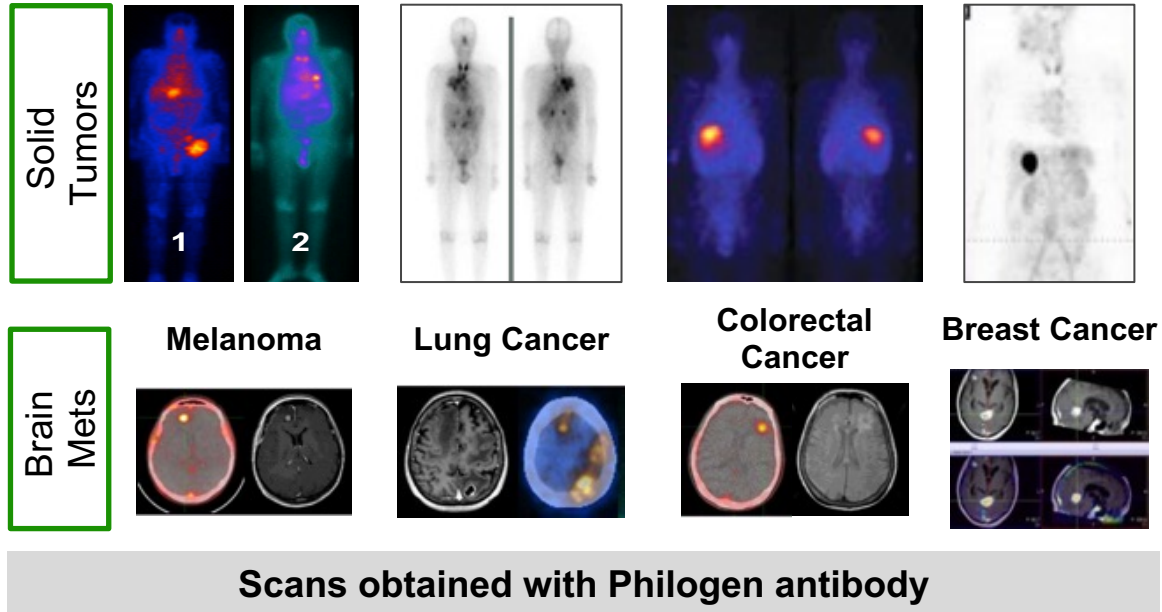




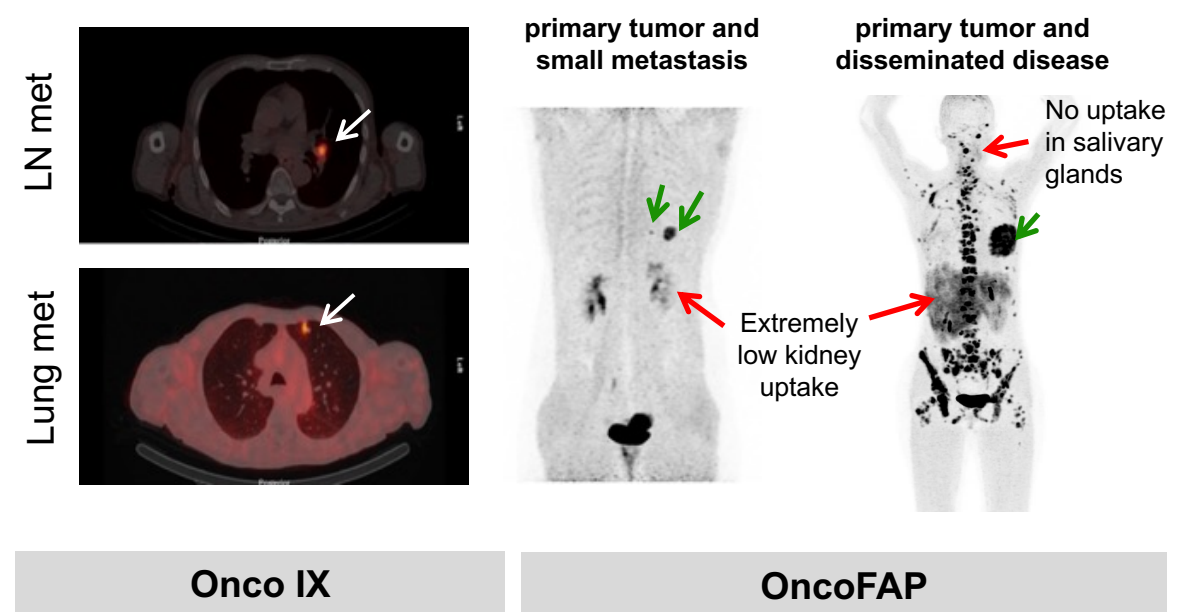
# Nuclear Medicine validation of tumor targeting



## Antibodies: Nuclear Medicine Validation in >200 Patients



## Small Molecules: Faster Delivery to Tumour Sites



- Identified ligands have a pan-tumoral targeting potential
- Proven targeting potential across antibodies and small molecules
- Strong Nuclear Medicine validation

Poli et al., *Cancer Immunol Res*, **2013**, 1, 134;

Brouwers et al., *Journal of Clinical Oncology*, **2005**, 23, 6540;

Palmer et al., *Neuroncol Adv*, **2020**, 2, vdaa034;

Kulterer et al., *J Nucl Med*, **2020**, 62, 360

# Example 1: a clinical-stage antibody-cytokine fusion



**Emanuele Puca, Ph.D.**  
Director glioblastoma project



**Teresa Hemmerle, Ph.D.**  
Director L19-TNF project

# Antibody-based cytokine delivery



## Cytokines Dramatically Modify the Tumor Environment

### Cytokine-induced tumor necrosis (e.g., TNF)

Baseline



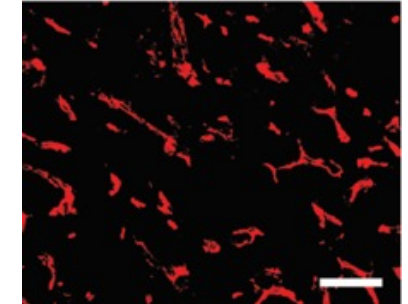
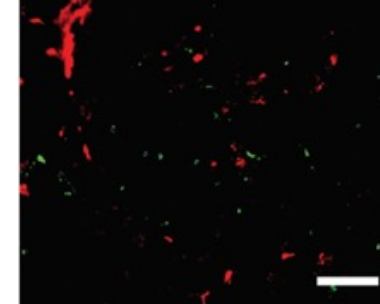
4h post injection



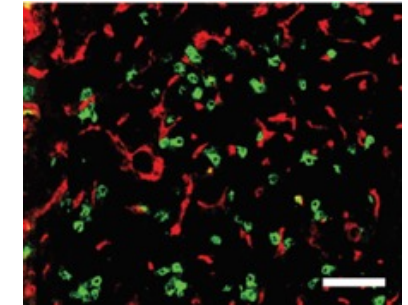
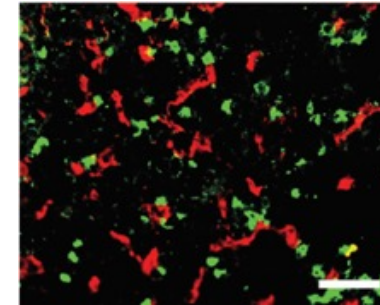
**TNF-based products induce selective hemorrhagic necrosis and thus act as “debulking” agents**

### Immuno-modulatory cytokines (e.g., IL12)

Baseline



24h post injection



**NK cells**  
**Blood vessels**

**CD8<sup>+</sup> T cells**  
**Blood vessels**

**IL12 based products promote the intratumoral elevation of immune cells which are crucial to eradicate cancer**

# Antibody-based delivery of tumor necrosis factor (TNF)

Targeted active ingredient...

...administered systemically...

...causes selective tumor necrosis

ANTIBODY

**L19 Antibody**

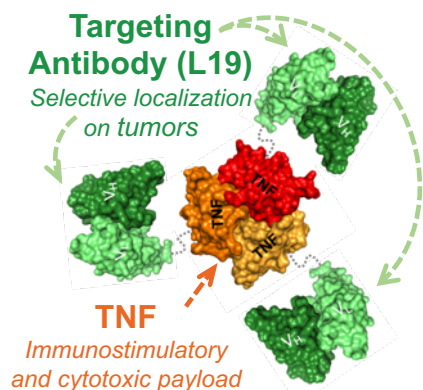
Selective localization to EDB(+) Fibronectin tumors

PAYLOAD

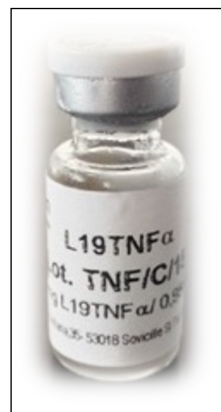
**Cytokine TNF**

Immune modulation & cytotoxicity

STRUCTURE

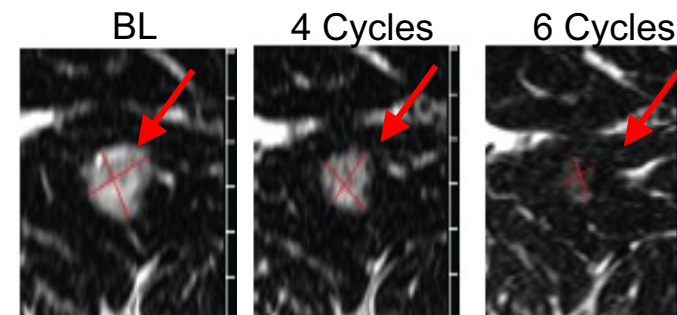


**FIBROMUN**

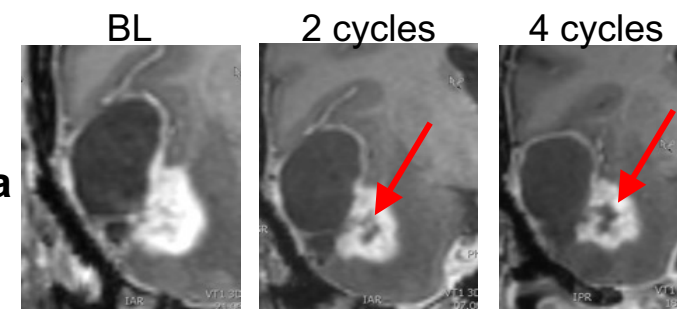


ODD Status both in EU and US

**Soft Tissue Sarcoma**  
Combo with Doxorubicin



**Recurrent Glioblastoma**  
Monotherapy

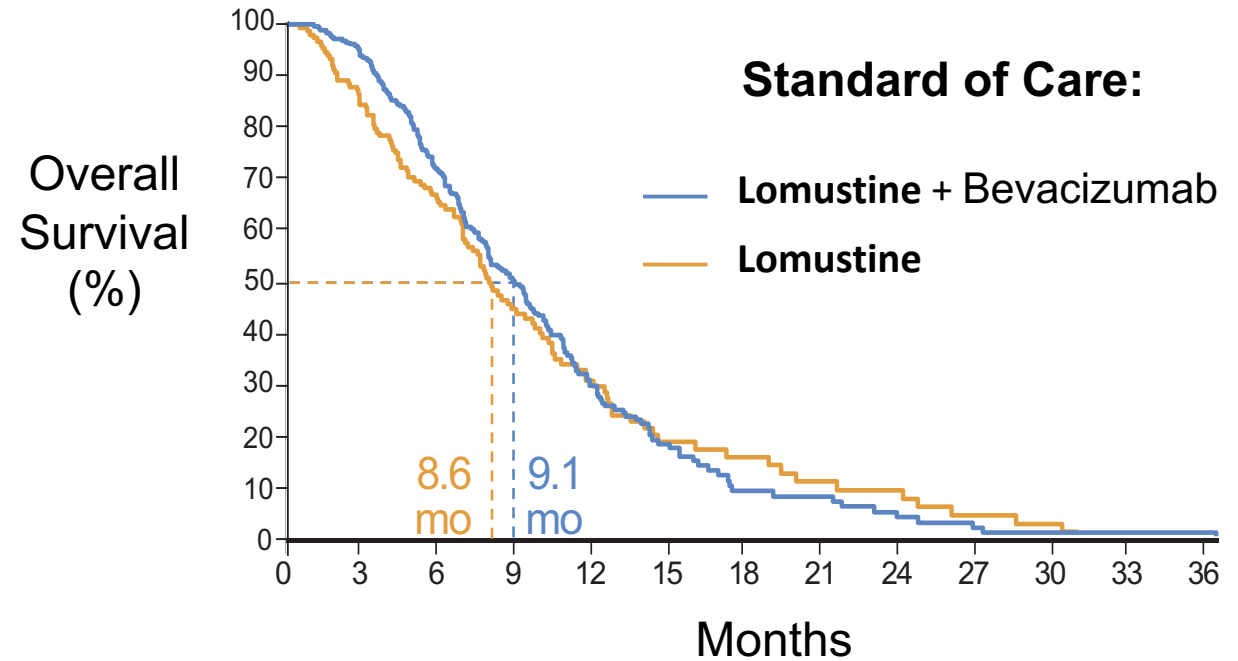


- Fully human protein produced in CHO cells; no immunogenicity
- Administered to > 300 patients alone or in combination
- Recommended Dose is 13 µg/kg (very well tolerated)

# Glioblastoma: the most aggressive brain tumor



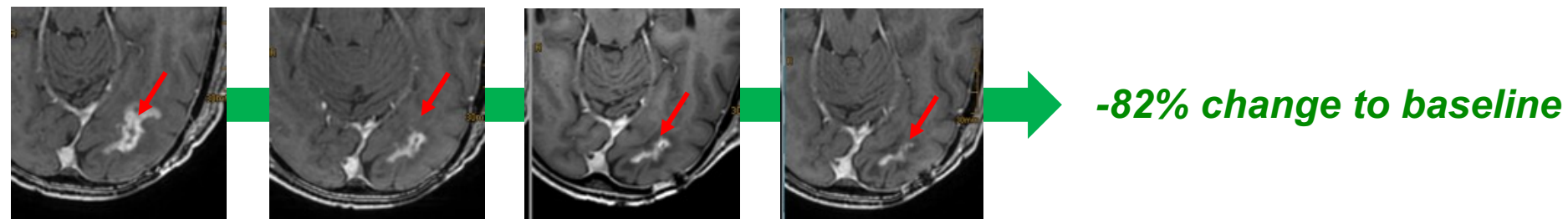
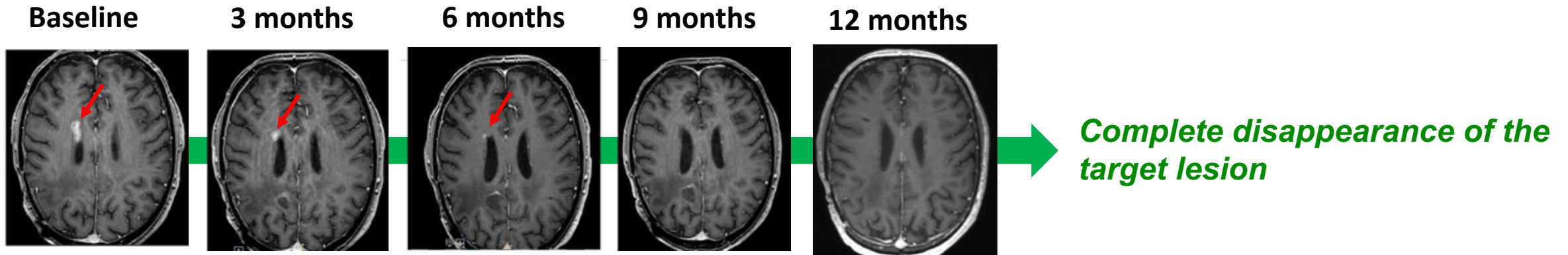
*Glioblastoma multiforme*



Second-line glioma patients with **unmethylated MGMT promoter** do not respond to standard drugs, **progress within 6 weeks** and typically **die within 6 months**

# Emerging results in glioblastoma (second-line)

Two patients from the first cohort of the clinical trial with Fibromun (L19-TNF) plus lomustine in second-line glioblastoma:



Prof. Dr. med.  
Michael Weller



Dr. med.  
Tobias Weiss



Strictly confidential

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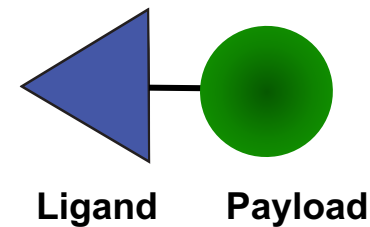
 **UniversitätsSpital  
Zürich**

# **Example 2:** **a small-molecule tumor targeting agent**

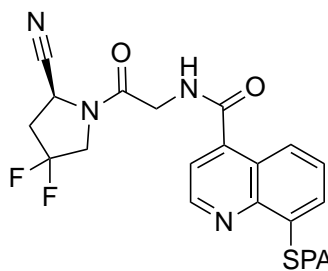
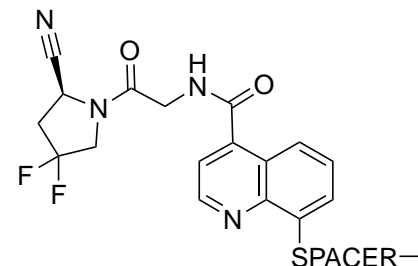


**Samuele Cazzamalli, Ph.D.**  
Director Small Molecule Therapeutics

# OncoFAP: a best-in-class FAP targeting agent

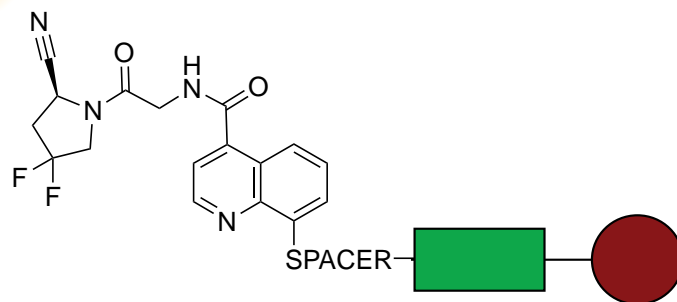
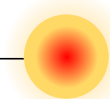


**OncoFAP**  
(Kd = 680 pM)



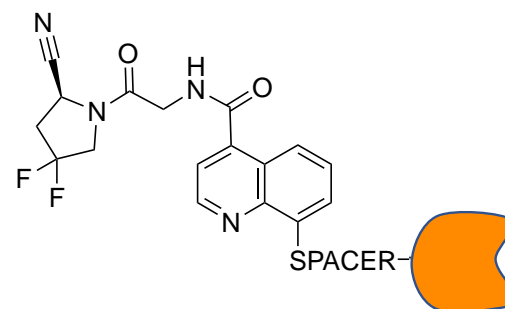
## Radio Conjugates

Backhaus et al., *Eur J Nucl Med Mol Imaging*, 2021, 10.21203/rs.3.rs-969176/v1

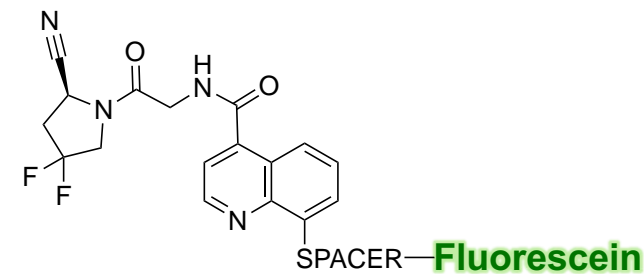


## Drug Conjugates

Millul et al., *PNAS*, 2021, 118, 16, e2101852118  
Zana et al., *submitted*  
Galbiati et al., *submitted*





## Bispecific Immuno-Modulators



## Adaptor for Universal CAR-Ts

Pellegrino et al., *Bioconj Chem* 2020, 31, 1775

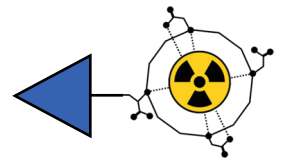
 = radiometal chelator (DOTA or DOTAGA)

 = linker-drug

 = small molecule immunomodulator



# Imaging performance of OncoFAP in cancer patients



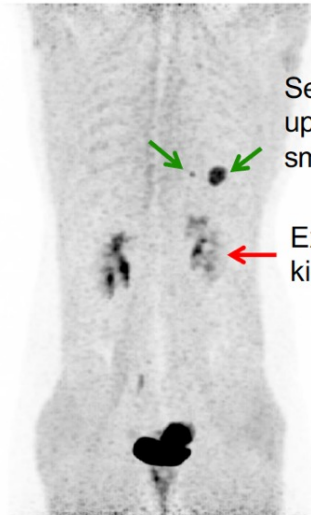
Breast cancer patients imaged with OncoFAP-<sup>68</sup>Ga (PET) at ~ 1h p.i.



No uptake in the salivary glands  
Selective uptake in tumor lesions  
Extremely low kidney uptake

Patient with a primary tumor only

t.p. = 1 h



Selective uptake also in small lesions  
Extremely low kidney uptake

Patient with a primary tumor and a small lymph node metastasis

t.p. = 1.3 h



Selective uptake in tumor lesions  
Extremely low kidney uptake

Patient with a newly diagnosed Metastatic breast cancer to local and supraclavicular lymph nodes

t.p. = ~1 h



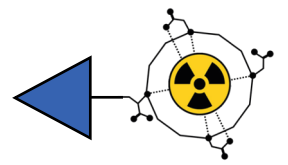
No uptake in the salivary glands  
Extremely low kidney uptake

Patient with a primary tumor and disseminated cancer lesions

t.p. = 1 h

- OncoFAP shows **excellent tumor targeting properties** not only in breast cancer, but also in other solid tumors
- Dosimetry supports **therapeutic applications** with therapeutic conjugates (i.e., OncoFAP-<sup>177</sup>Lu)

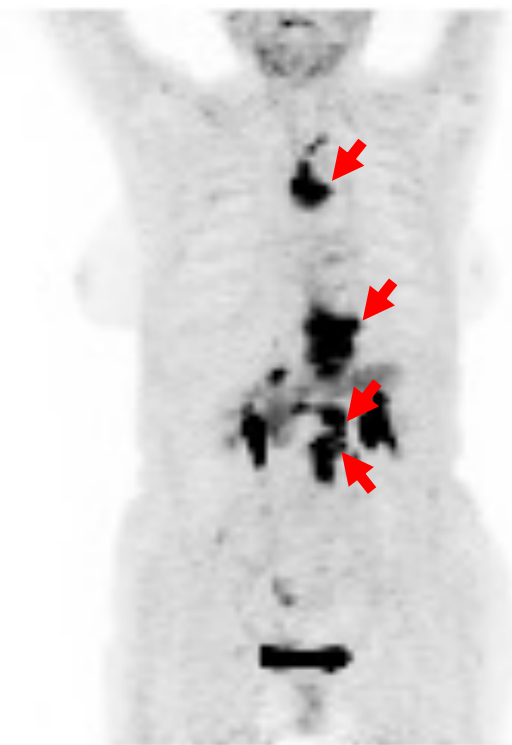
# Imaging performance of OncoFAP in cancer patients



Comparative performance of  $^{18}\text{F}$ -FDG and OncoFAP- $^{68}\text{Ga}$  (PET) in esophageal cancer

$^{18}\text{F}$ -FDG-PET

$^{68}\text{Ga}$ -OncoFAP



*Patient with metastatic esophageal cancer*

- OncoFAP appears to be superior to  $^{18}\text{F}$ -FDG for the detection of metastatic lesions in esophageal cancer

# Some (personal) conclusions

- In Italy, we (still) have many **talented young scientists**, who however need **adequate training**
- **Role models** are important
- In the healthcare sector, **simple changes** to certain (stupid) regulations could have an **immediate beneficial effect**
- **Disruptive technologies** (e.g., encoded libraries) are revolutionizing the way **drugs** are being **discovered** and **developed**