ANR-15-IDEX-0003

1ST ISITE-BFC SEMINAR

BIOCAIR Biomarkers of T-Cell Activity in tumors and Immunotherapy Response

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CLINICAL CONTEXT

1. Recently, immunotherapies using monoclonal antibodies targeting checkpoints inhibitors of the immune response, such as PD-1, demonstrated their superiority compared to standard chemotherapies
2. However, despite the strong contribution of anti-PD-1 (nivolumab) in some cancer treatment, this immunotherapy is not effective in all patients

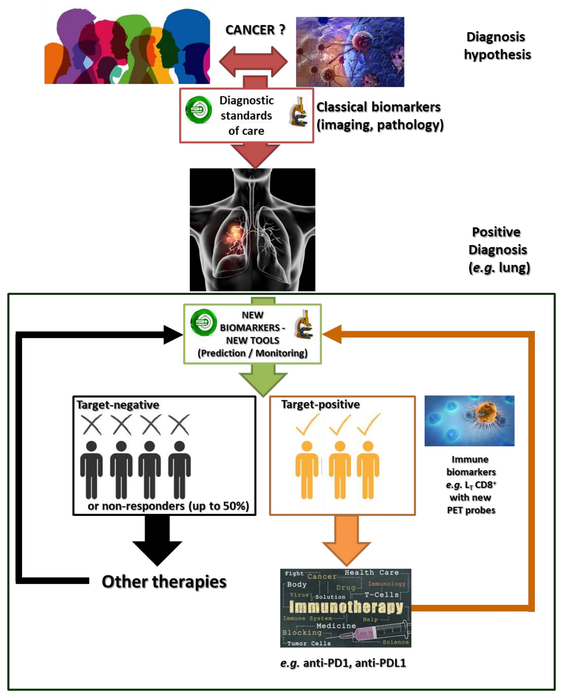
→ **Predictive biomarkers of treatment efficacy are needed for efficient patient management**

→ **Recent translational studies suggested that the level of tumour infiltrated CD8 T Lymphocytes is a good biomarker to predict immunotherapy efficacy**

In this context, the aims of the BIOCAIR project are:

GOALS

- WP1 **To obtain a proof of concept of the use of radiolabelled anti-CD8 fragments as imaging biomarkers of the efficacy of immunotherapies**
- WP2 **To identify at least 3 new biomarkers as potential biomarkers of immunotherapy efficacy**
- WP3 **To develop antibodies against these targets and validate them as imaging biomarkers of immunotherapy effectiveness**



KEY NUMBERS

2018

6 partners

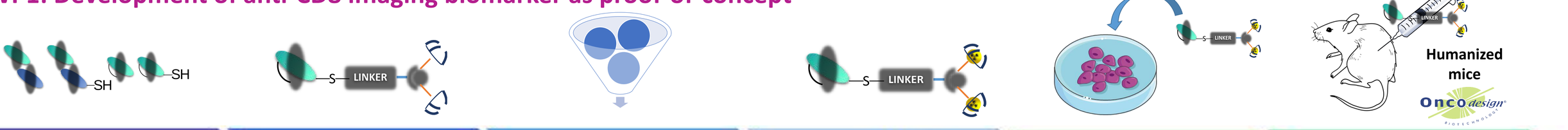
1.3 M€

6 funded positions

PHARM'IMAGE Platforms and competences for pharmacology

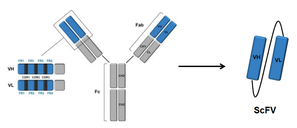
Twice monthly coordination meetings

1. WP1: Development of anti-CD8 imaging biomarker as proof-of-concept



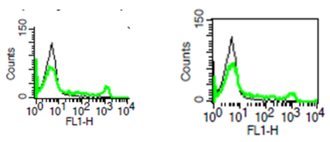
CD8 FRAGMENTS PRODUCTION

1. Clone sequencing and reformatting in ScFv



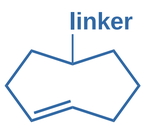
2. Validation of recombinant format by flow cytometry

Full ScFV

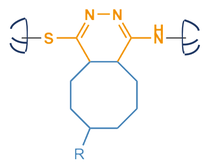


SYNTHESIS OF CONJUGATES

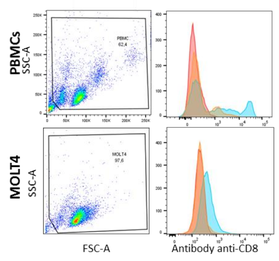
1. Random or site-specific conjugation with **TCO bearing linker**



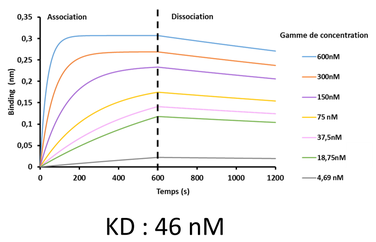
2. Inverse Electron Demand Diels-Alder with **Tetrazine platform**



FLOW CYTOMETRY

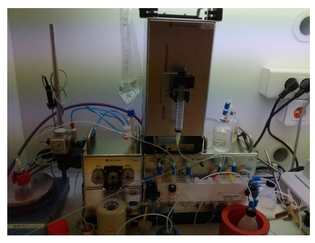


BIO-LAYER INTERFEROMETRY



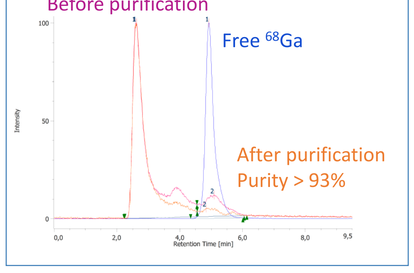
KD : 46 nM

Ga68 PROTOCOL & QC



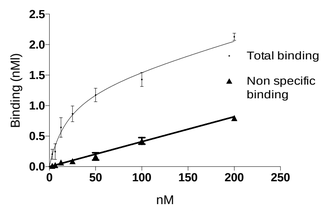
SIZE-EXCLUSION RADIOCHROMATOGRAPHY

Before purification

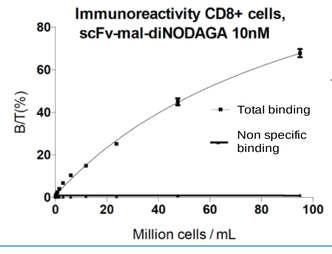


BINDING SPECIFITY

CD8+ cells affinity, scFv-mal-diNODAGA



K_D = 20,83 ± 4,53 nM
Bmax = 1,364 ± 0,108 nM

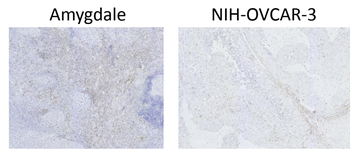


CD8+ T CELL PET IMAGING

Selection of model

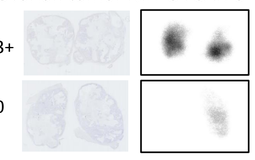
Immunochemistry of CD8 tumor infiltrate

Positive control Amygdale | Ovarian tumor NIH-OVCAR-3



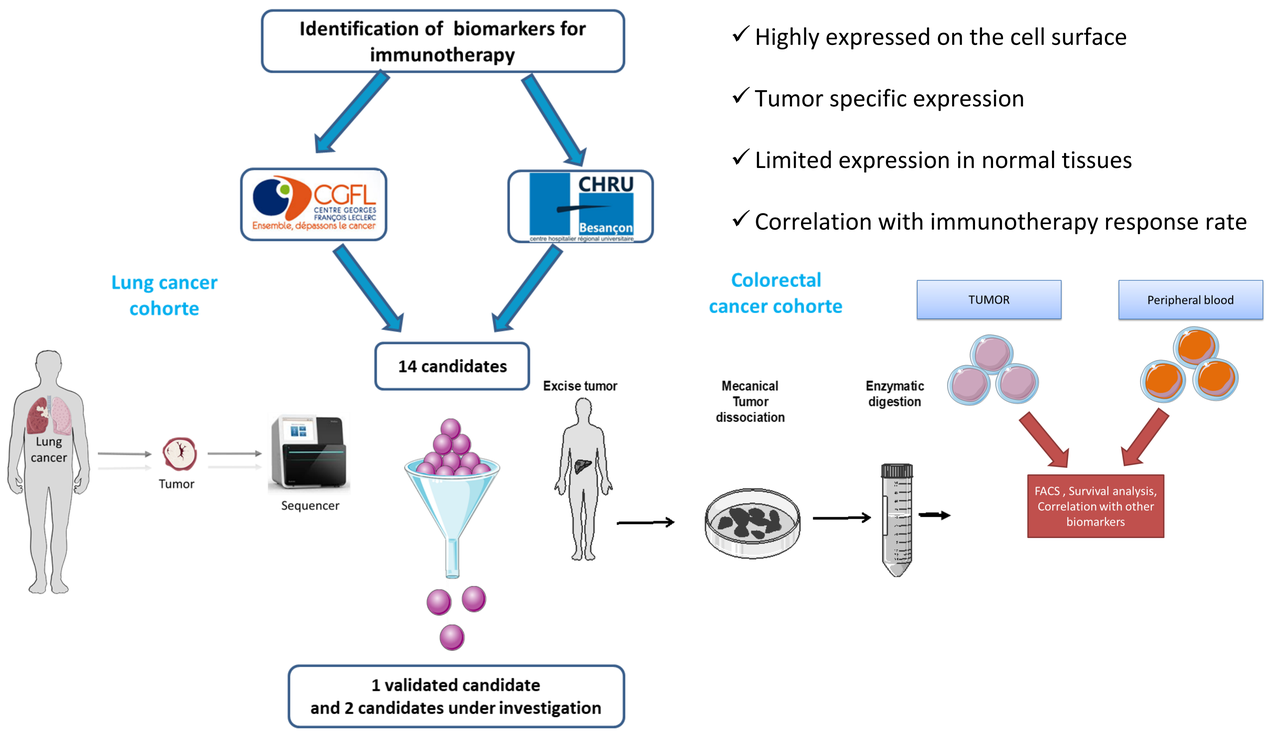
Autoradiography

Ovarian tumor NIH-OVCAR-3



Next Step: *in vivo* PET imaging of Ga68-diNODAGA-scFv-CD8

WP2: Identification of new biomarkers

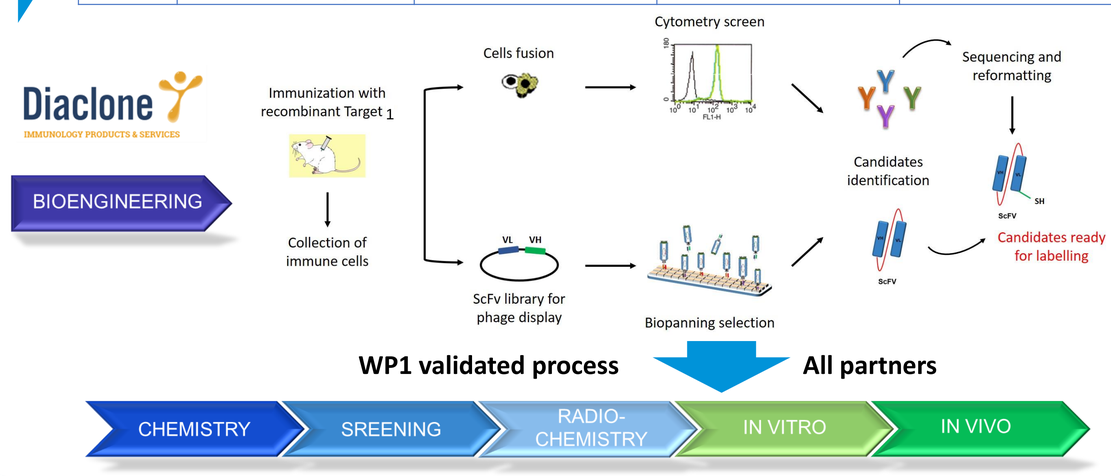


Properties of validated biomarkers:

- ✓ Highly expressed on the cell surface
- ✓ Tumor specific expression
- ✓ Limited expression in normal tissues
- ✓ Correlation with immunotherapy response rate

WP3: Development of new imaging biomarkers

Target	Tumor expression	Immunotherapy biomarker	Favorable for imaging	Preclinical model
CD8	☑ Lymphocytes T	☑ Survival	☑ High expression	☑ Humanized model
1	☑ Stroma	☑ Survival	☑ Selective tumor Expression	☑ Xenograft
2	☑ Lymphocytes B	☑ IT response	☑ High expression	☒ To be identified
3	☑ Monocytes/ Macrophages	☑ Survival	☑ High expression	☒ To be identified



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Conclusion

1. The BIOCAIR project is a **successful combination of translational skills and multidisciplinary know-how** of several UBFC laboratories, hospitals, biocluster and biotech companies from the Bourgogne Franche-Comté region
2. These developments will be **valorized with novel intellectual properties, scientific publications, new commercial opportunities** for Diaclone's antibodies, and the preclinical proof of concept of new biomarkers for further clinical application