1ST ISITE-BFC SEMINAR

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

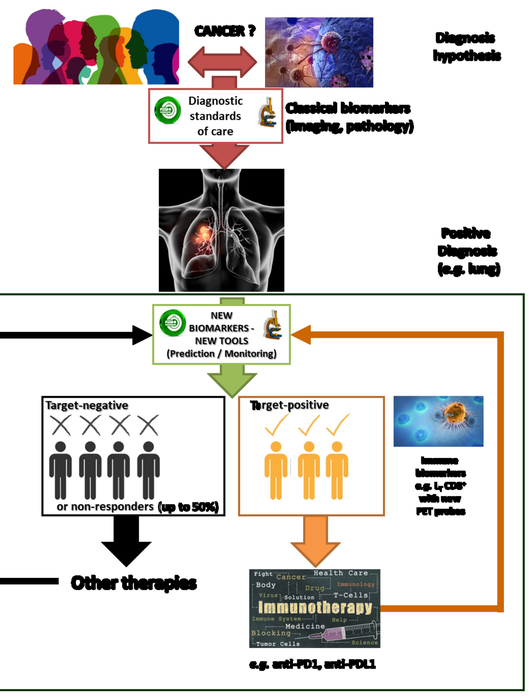
Predictive biomarkers of treatment efficacy are needed for efficient patient management

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

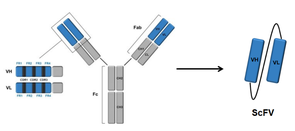
PHARM'IMAGE Platforms and competences for pharmacology

Twice monthly coordination meetings

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

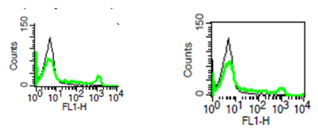
CD8 FRAGMENTS PRODUCTION

1. Clone sequencing and reformatting in ScFv



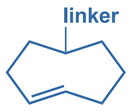
2. Validation of recombinant format by flow cytometry

Full ScFV

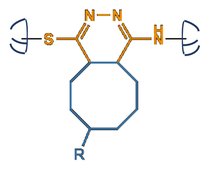


SYNTHESIS OF CONJUGATES

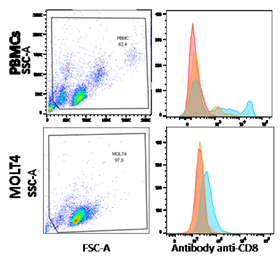
1. Random or site-specific conjugation with TCO bearing linker



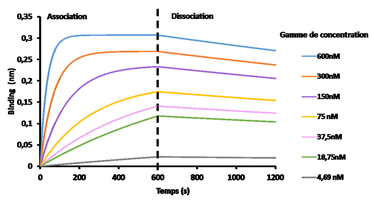
2. Inverse Electron Demand Diels-Alder with Tetrazine platform



FLOW CYTOMETRY

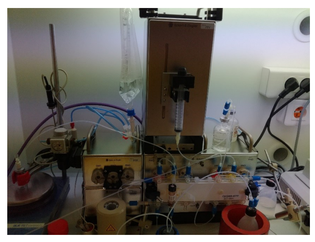


BIO-LAYER INTERFEROMETRY

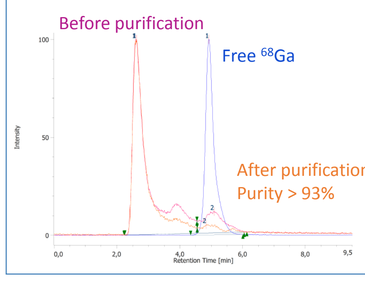


KD : 46 nM

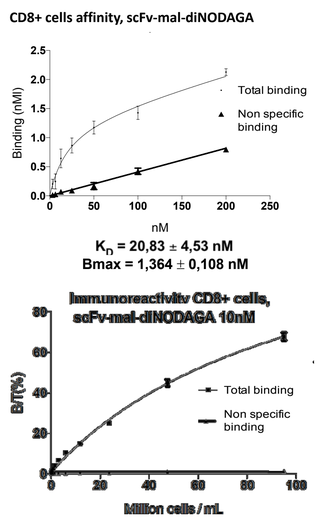
Ga68 PROTOCOL & QC



SIZE-EXCLUSION RADIOCHROMATOGRAPHY



BINDING SPECIFITY



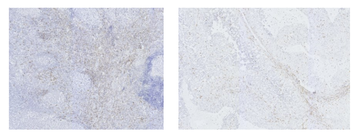
K_D = 20,83 ± 4,53 nM
Bmax = 1,364 ± 0,108 nM

CD8+ T CELL PET IMAGING

Selection of model

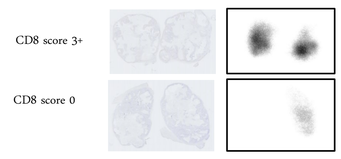
Immunochemistry of CD8 tumor infiltrate

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:

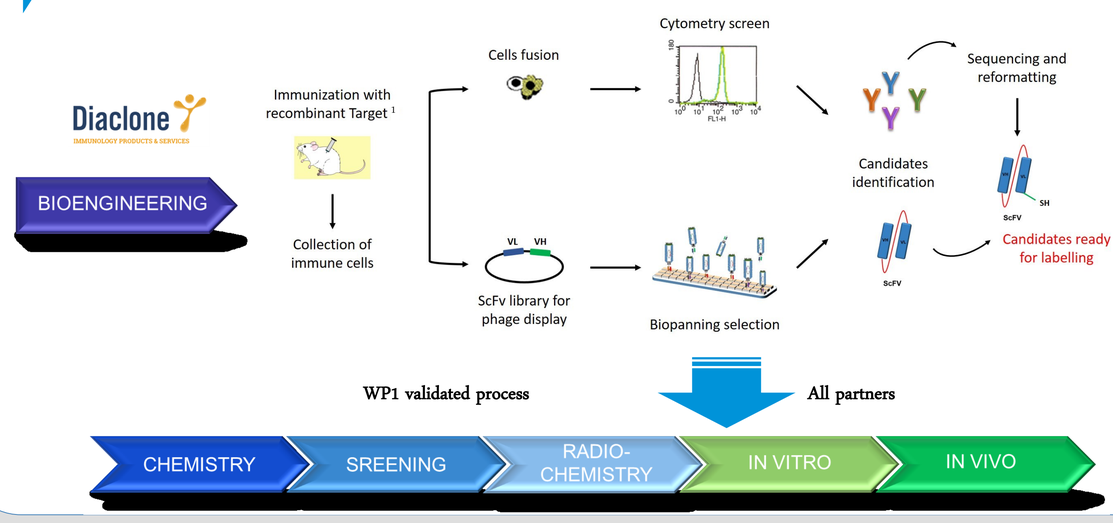
ice

ues

response rate

WP3: Development of new imaging biomarkers

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