RECOMBINANT ANTIBODIES FOR RESEARCH, DIAGNOSIS AND THERAPY

WITH BIOLOGICAL *IN VITRO* (CELLS) & *IN VIVO* (ANIMAL) CHARACTERIZATION

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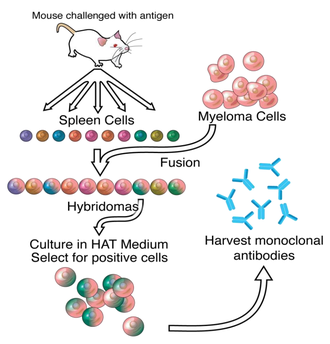
ABSTRACT:

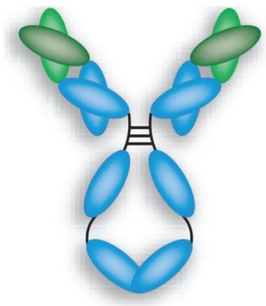
Antibodies are promising candidates for basic research, diagnosis and treatment. Currently, multiple monoclonal and recombinant molecules recognizing tumor targets are in preclinical o clinical assays. Accordingly, innovative ones, biosimilars or recombinant fragments with enhanced functions are continuously evolving the state of the art in the field. Our laboratory has a great expertise and experience in antibodies with a clear therapeutic focus aimed principally to oncology. Several examples are: 1) We have generated a number of neutralizing monoclonal antibodies against several members of the S100 protein family; we also proved their therapeutic efficacy in house using particular cellular and animal models leading to the inhibition of tumor growth, tumor metastasis, and tumor angiogenesis in immunodeficient mouse xenograft models of colon, melanoma, pancreatic and other human cancers. 2) We have obtained chimeric and humanized versions of these molecules as clinical candidates. 3) We have generated and characterized several biosimilar antibodies such as anti-VEGF molecules. 4) We have created site-specific linking ADCs with enhanced cytotoxicity over tumor cells. 5) We have started new projects involving nanobodies, bispecific antibody fragments and other recombinant multimeric molecules. It is also important to highlight that in order to characterize all these new monoclonal antibody formats at the analytical, immunological and biological function levels we have two broad in house platforms of in vitro cellular assays and in vivo animal models. LEITAT Biomed invites you to collaborate with us in European projects, services or other platforms in different industrial sectors such as human and veterinarian health, food and environment.

MONOCLONAL ANTIBODIES



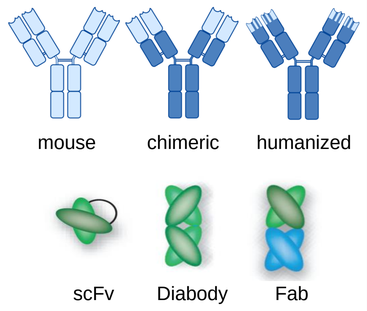
- Hybridomas
- Mouse and rabbit polyclonals
- Isotyping & Characterization
  - Generation of Fab and F(ab')2 fragments
  - Labelling (biotin, fluorochrome…)

- Transient expression
- Stable clones
- Production & Purification



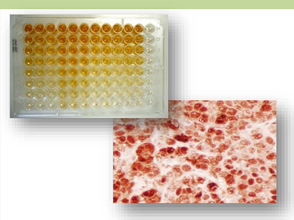
ANTIBODY ENGINEERING

- Sequencing of variable regions
- Generation of Chimeric and Humanized mAbs
  - Recombinant fragments (scFv, Fab)
  - Fusion proteins & ADC
  - Bispecifics
  - Nanobodies
- Generation of biosimilar antibodies



ANTIBODY CHARACTERIZATION

In vitro assays



- ELISA
- Western-blot
- Flow cytometry
- ADCC, CDC
- Viability
- Proliferation
- Apoptosis
- Nanotox
- Immunohistochemistry
- Migration
- Adhesion
- Target validation by siRNA
- Arrays, pharmacogenomics, transfection

Efficacy assessment
Mechanism of action
Drug reprofiling
Preclinical studies
Biodistribution

In vivo models

- Syngenic & xenogenic
- Subcutaneous & orthotopic
- Metastasis (experimental & spontaneous)
- Tumor explants from clinics (PDXs, Tumorgrafts)
- Tumor angiogenesis
- Angiogenesis in Matrigel plugs
- Multiple cancers

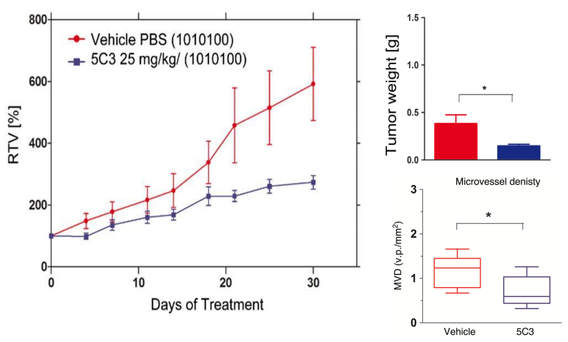
- Non-invasive imaging

ONGOING PROJECTS

Therapeutic antibodies

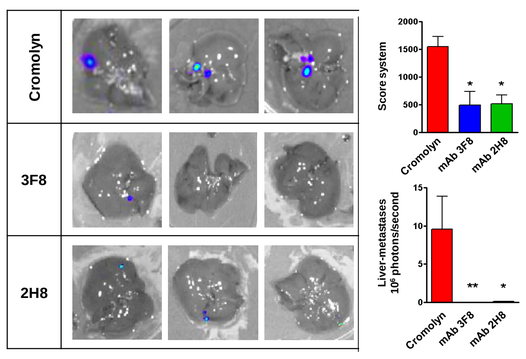
Therapeutic targeting of tumor growth and angiogenesis with anti-S100 mAbs

Anti-S100A4 mAb 5C3 reduces MiaPACA-2 tumor growth and angiogenesis



Bars of tumor weight show the mean ± SEM. **p<0.05.* Quantification of density and area fraction of CD31 positive vessels in Mia PACA-2 tumors after 30 days of treatment with mAb 5C3 or PBS. *Mann Whitney U-test *p<0.05.*

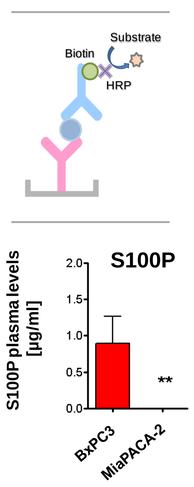
Anti-S100P mAbs 3F8 and 2H8 reduce liver metastasis formation in an orthotopic BxPC3-luciferase tumor model



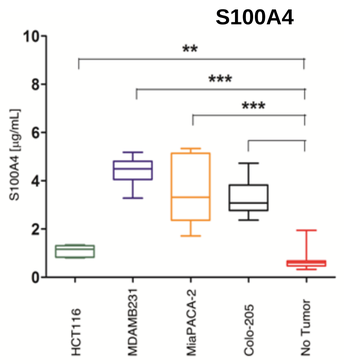
Score system according to a TMPN classification and photon emission quantification of liver metastases. *Mann Whitney U-test * p<0.05, ** p<0.01.*

mAbs as diagnostic tools

Sandwich ELISA quantification of promising plasmatic biomarkers

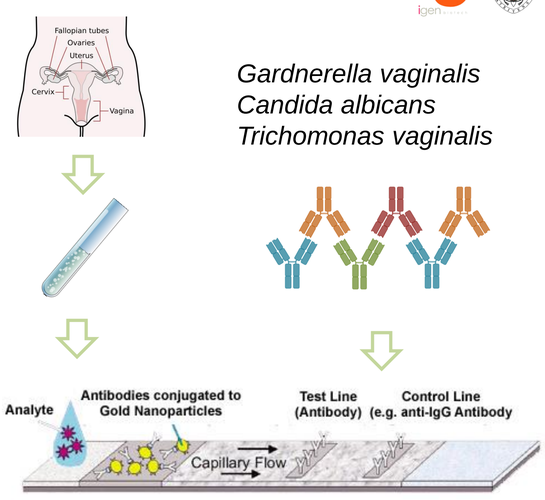


BxPC3 (S100P +/+)
MiaPACA-2 (S100P -/-)

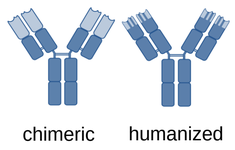


Sandwich ELISA quantification of S100 plasma levels in mice bearing tumors of the indicated tumor cell lines. Graph shows mean ± s.d. *Mann Whitney U-test ** p<0.01.*

Development of a multi-diagnostic kit by lateral flow immunoassay to detect vaginal infections



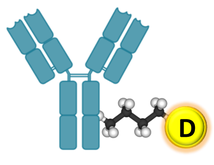
Humanized antibodies



Development of chimeric and humanized variants (CDR grafting) of potential therapeutic antibodies have been developed. Preclinical assays are now ongoing.

Antibody Drug Conjugates (ADC) – Toxab project

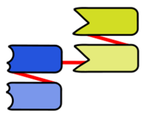
Application of ProteoDesign's Streamlined Expressed Protein Ligation technology (sEPL) to develop new ADC molecules for cancer treatment



- Prevents loss of activity of the antibody
- Enables the use of more potent cytotoxic drugs without risk of premature release
- Enables the incorporation of more than one type of cytotoxic

ProteoDesign

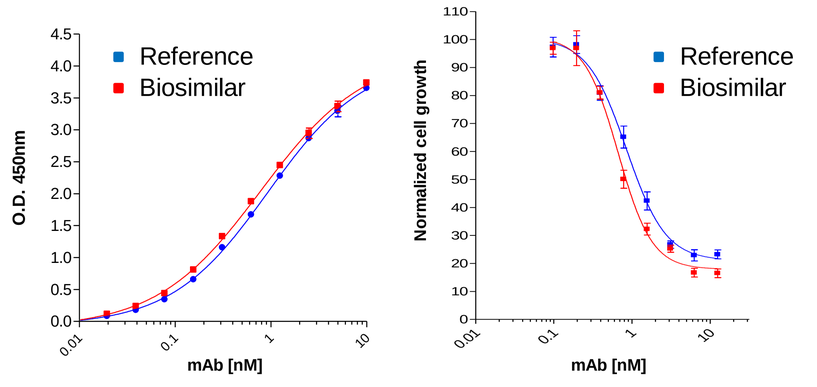
Immunotherapy with bispecifics



New projects include engineered bispecific antibodies, monovalent and multivalent variants to provide antibodies with novel functionalilties, optimized half-life and better tumor penetration.

Biosimilar antibodies

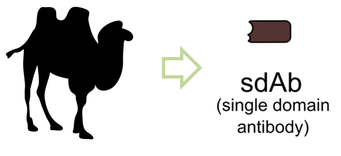
Development of high efficient expression systems to produce biosimilar antibodies in mammalian cells. Development of biosimilar anti-VEGF mAb.



A) Comparison of antigen binding between biosimilar and reference antibodies by ELISA.

B) Inhibition of proliferation of biosimilar and reference antibodies analyzed by Alamar blue assay.

Single domain antibodies (Nanobody)



New single domain antibody libraries are ongoing. Those small molecules have the advantage to block hidden epitopes with higher stability and higher tumor penetrability than whole antibodies.

COLLABORATIONS

GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION HORIZON 2020
ACC1Ó Competitivitat per l'empresa · Generalitat de Catalunya
LYKERA biomed

PATENTS

WO/2011/157724: S100A4 antibodies and therapeutic uses thereof
WO/2012/098124: Antibodies against the S100P protein for the treatment and diagnosis of cancer
WO/2014/167030: Anti-S100A7 antibodies for the treatment and diagnosis of cancer