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GROUP 5

BIOMEDICAL ENGINEERING
MOLECULAR BIOLOGY FOR ENGINEERS
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DEVELOPMENT OF A MONOCLONAL ANTIBODY TARGETING SICAM-1 TO ENHANCE THE EFFICACY OF IMMUNOMODULATORY THERAPIES IN MELANOMA TREATMENT

1 PROBLEM IDENTIFICATION

Cell adhesion is crucial for immune regulation and cancer progression. In the tumor microenvironment, interactions between tumor cells and the immune system drive invasion, metastasis, and immune evasion. Adhesion molecules like ICAMs facilitate immune cell migration and activation at the tumor site. Overproduction of sICAM-1 (soluble ICAM-1) disrupts these interactions, promoting immune evasion and treatment resistance by blocking immune cell adhesion and migration to the tumor

How does the overproduction of sICAM-1 in melanoma contribute to tumor immune evasion and resistance to treatment, and how can targeting sICAM-1 improve the efficacy of immune-modulating therapies for melanoma?

THE ROLE OF SICAM-1 IN MELANOMA

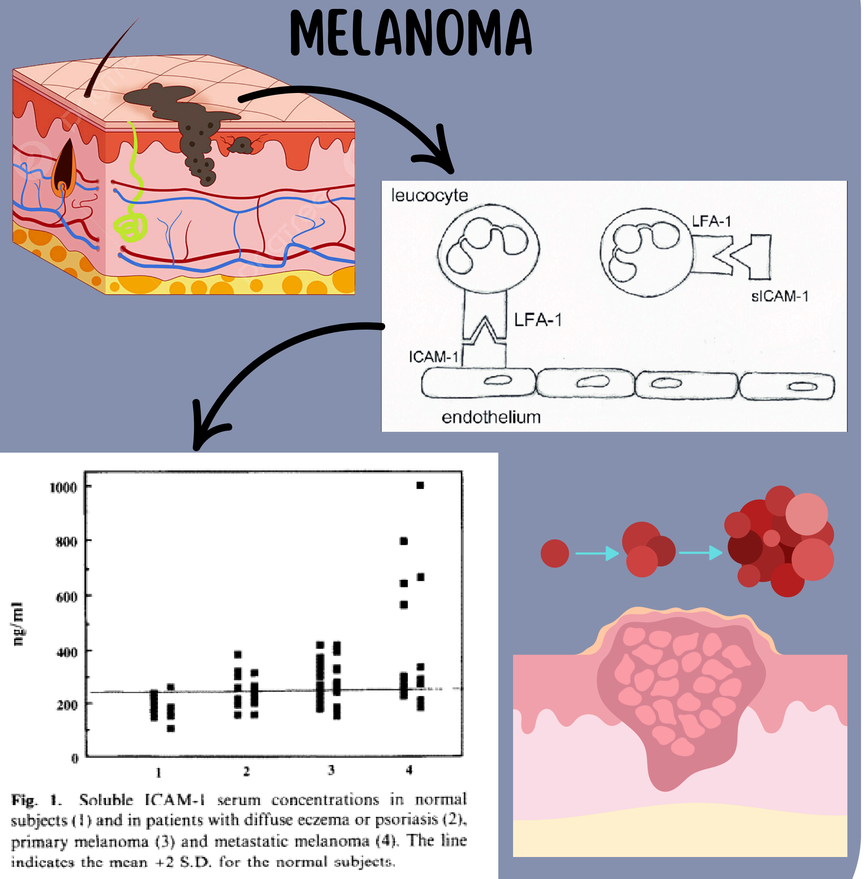


Fig. 1. Soluble ICAM-1 serum concentrations in normal subjects (1) and in patients with diffuse eczema or psoriasis (2), primary melanoma (3) and metastatic melanoma (4). The line indicates the mean +2 S.D. for the normal subjects.

2 OBJECTIVE

Design and development of a monoclonal antibody targeting sICAM-1 to inhibit its function as a molecular decoy, aiming to restore immune recognition and optimize melanoma treatment through a combined strategy that addresses tumor resistance pathways and enhances the immune response against the tumor

SPECIFIC OBJECTIVES

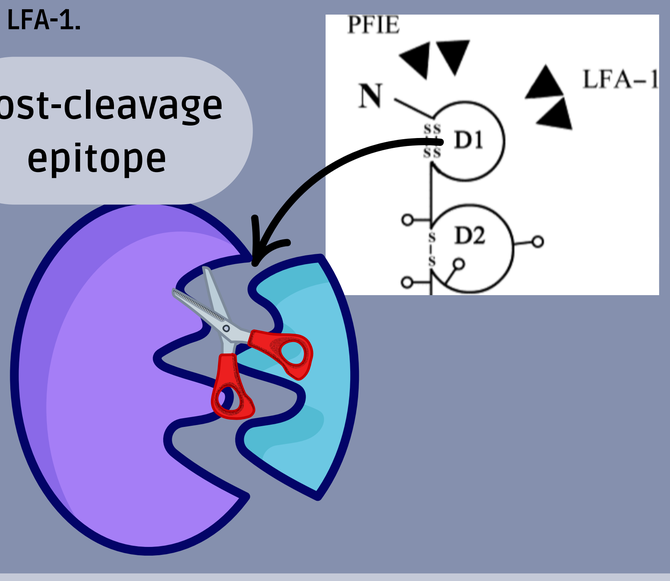
- Identify molecular factors driving sICAM-1 overproduction in melanoma tumors.
- Design a monoclonal antibody to inhibit sICAM-1.
- Evaluate the antibody's effect on tumor immune evasion and resistance, and its potential to improve melanoma treatment efficacy.
- Develop and assess a combined therapeutic approach.

3 STRATEGY

"What do we need to know?"

The key interaction between ICAM-1 and LFA-1 occurs mainly in the D1 domain of sICAM-1. However, directly blocking this domain may not always be effective or could interfere non-specifically with other ICAM-1 interactions. Therefore, instead of focusing only on the D1 domain, we can explore other sites, like the post-cleavage epitope, which do not directly bind but may alter sICAM-1's conformation, affecting its interaction with LFA-1.

post-cleavage epitope



- Proximity to the Functional Domain (D1)
- Competitive Blockade and Conformational Alteration

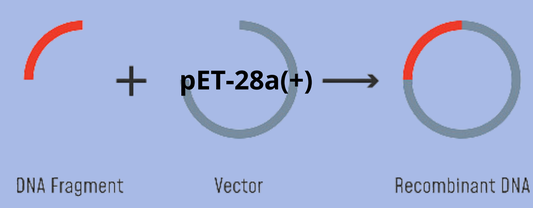
4 EXPECTED RESULTS

- Development of a Specific Monoclonal Antibody: Restoration of Immune Response, Reduction in Tumor Growth, Compatibility for Clinical Use
- Better Understanding of sICAM-1/LFA-1 Interaction
- Validation of Antibody Specificity and Efficacy

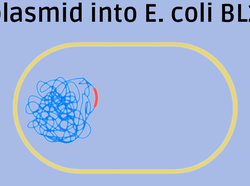
MOLECULAR STRATEGY PROCESS

Sequence to use: **ACG CAA GGT CCG GGT GCA AAC CTG ACT CTG CGT TGC GAG GTT TCT TTT CCG AAA AAC CTG ACG GAG CTG TCT AAA CAA AAA CTG TCT TTT**

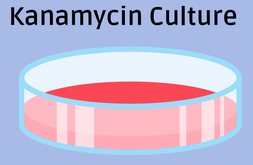
Digestion of the Epitope and the pET-28a(+) Vector



Insertion of the recombinant plasmid into E. coli BL21

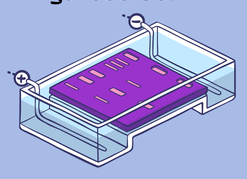


Selection of Effective Plasmids with Kanamycin Culture

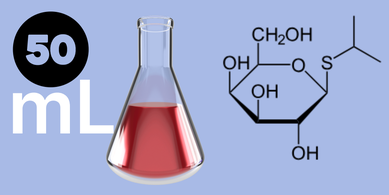


Colony PCR

Confirmation of Bands in Agarose Gel

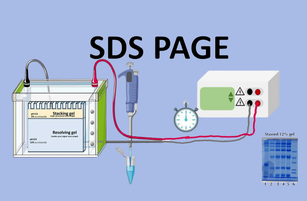


Expression and Purification with IPTG



Purification by Affinity Chromatography

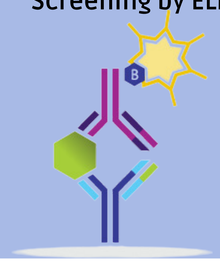
Verification by SDS-PAGE and ELISA



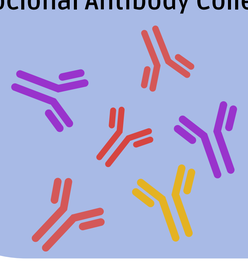
Immunization of Mice and Monitoring of Immune Response

Obtain hybridomas, which produce monoclonal antibodies against the post-cleavage epitope of sICAM-1.

Screening by ELISA



Monoclonal Antibody Collection



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