Developing Functional Monoclonal Antibodies for Beta 3 Adrenergic Receptor

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Abstract
G-protein-coupled receptors (GPCRs), one of the most commonly used and successful targets for drugs, are a large family of multi-transmembrane proteins and an important class of receptors. Over 40% of all modern medicines interact with this protein group. These cell surface receptors are acted on by a wide variety of ligands, including small molecules and soluble proteins. Monoclonal antibody (mAb) therapy has major advantages over small molecule therapy in that mAbs are more selective and therefore tend to have fewer non-specific or off-target toxicity issues, while having a longer duration of action than small molecule drugs. Unfortunately, it is extremely difficult to create antibodies against GPCRs using traditional approaches, especially for clinical applications. Multispan combines its proprietary immunization technology using its patented GPCR high expression system and in-depth expertise in developing well-designed and validated GPCR functional assays to select mAbs that perturb disease-relevant signaling pathways. In this poster, we detail the development of Beta-3 adrenergic mAbs. Multispan has demonstrated specific target GPCR binding shown in Figure 1 and 2.

Rationale for [β3-AR] Monoclonal Antibody Therapeutics
[β3-AR] as a cardiovascular disease target:
- Cardiac hypertrophy elicited by positive inotropic cardiac protective signaling
- Expression up-regulated in disease conditions such as ischemia in both rats and humans.
- Mediates the activation of endothelial nitric oxide synthase (eNOS) and increase of nitric oxide (NO) levels that in turn protect the heart against ischemia-perfusion injury and improve survival after heart attack in animals and humans.
- The potential therapeutic indications of [β3-AR] agonists are early heart failure, ventricular hypertrophy, ischemic heart disease in addition to hypertension.

Problem: [β3-AR] small molecule agonists cross-reacted with [β1-AR] and [β2-AR], causing significant side effects.
Solution: mAb approach is an attractive alternative due to mAbs' known superior specificity.

Polyclonal Antibody Bound to [β3-AR] Selectively
Figure 2. Multispan polyclonal antibody selected in mice selectively bound to [β3-AR] in native membranes and successfully expressed GPCRs expressed in the same HEK297 transfection cell background. HEK297 cells stably expressing [β3-AR] and cell lines [5] and [6] were used to evaluate the polyclonal specificity. Anti-FLAG antibody (Blue lines), mice polysera bound to Multispan β3-AR-over-expression cells with [β1-AR], [β2-AR] and after the 3rd immunization. Figure 3 shows the polysera staining of the [β3-AR] receptor with a specificity for [β3-AR] as determined by using a PE-conjugated mouse IgG secondary Ab. After the 1st immunization (pink lines) and after the 3rd immunization (blue lines) in primary staining prior to using a PE-conjugated and mouse IgG antibody in secondary staining. Positive results from 3 independent mice are shown (#1, #2, and #3) with #3 mouse showing the strongest immune response.

mAbs Bound to [β3-AR] with mM Affinity
Figure 3. Recombinant β3-AR-mAbs – mAb 1 and mAb 2 Antibody clones [β3-AR] over-expression cells with [β1-AR] and [β2-AR] activity as shown by flow cytometry using surface detection of the mAbs. [β3-AR] is a receptor for cAMP. The mAbs were engineered from scFv single chain fragment variable genes isolated from phage display libraries constructed from spleen of a GPCR immunized mouse at Multispan that demonstrated specific target GPCR binding shown in Figure 1 and 2. This work was done in collaboration with Dr. James Marks at UCSF.

Work in Progress
1. mAbs will be humanized, affinity matured, produced recombinantly and scaled up.
2. In vitro characterization: 1) functional specificity over [β1-AR] and [β2-AR]; 2) Gsa agonist signaling and hypertrophy protection in cardiomyocytes in vitro models.
3. In vivo characterization in wild-type and genetic-deficient mice: 1) [β3-AR]-control mice; 1) hypertrophy protection 2) cardiac remodeling.

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Preliminary Functional Data Showing [β3-AR] Agonist Activity
Figure 4. An example of functional agonist [β3-AR] mAbs. Spleen from an immunized mouse showing positive hetero- to the target was harvested and bioreactors (Sigma, Aldrich) were used for expression of [β3-AR] mAb. Spleen from an immunized mouse showing positive hetero- to the target was harvested and bioreactors (Sigma, Aldrich) were used for expression of [β3-AR] mAb. Spleen from an immunized mouse showing positive hetero-

Polysera + PE-conjugated mouse IgG secondary Ab

References
• Calvani M, Corsini A, Antonacopoulos GA, Missouri R, Heidrich L, Schindler AL, Beckers H, Schlicker A, Koster H, and Kung H. Evidence for a β3-Adrenergic Receptor Agonist of the 1084771 (3,2-β3-AR) myeloma fusion partner cells to make hybridomas. Purified mAbs derived from a hybridoma subclone demonstrated agonist activity to increase [3H]-dihydroalprenolol (β3-AR) binding. The assay was performed using Multispan stable cell line expressing the target GPCR selected using its natural agonist signal (data not shown).

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