

Engineered Antibody-Based Molecules for the Treatment of Ovarian Cancer

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Antibody-based Cancer Therapeutics

- **Target antigens can be tumor-associated or tumor-specific**
- **Target antigens are typically highly overexpressed in cancer cells**
- **The antibodies can function directly (naked) or serve as a vehicle for a cytotoxic agent**

Tumor-Associated Antigens

- Are the most common targets for antibody therapy
- Are typically expressed at low levels in normal tissues and at significantly higher levels in tumors (often 100,000 to 1,000,000 copies per cell)
- Examples include:
 - EGFR
 - HER2
 - CD20
 - etc.

Tumor-Specific Antigens

- These extremely rare targets are our field's "Holy Grail"
- Tumor-specific antigens are absent on normal tissues and expressed or overexpressed on tumor cells
- Examples include:
 - clonal surface Ig found on some B-cell lymphomas
 - EGFR vIII, an in-frame deletion mutation found in gliomas and other tumors

Therapeutic Mechanisms

Mechanisms of Antibody-based therapy

- Initiating signaling (e.g., inducing apoptosis with anti-TRAIL receptor antibodies)
- Inhibiting signaling (e.g., blocking EGFR ligand binding)
- Focusing the immune response (ADCC/CDC)
- Acting as a carrier for a cytotoxic agent (e.g., drugs, toxins, radioisotopes)

Ovarian Cancer

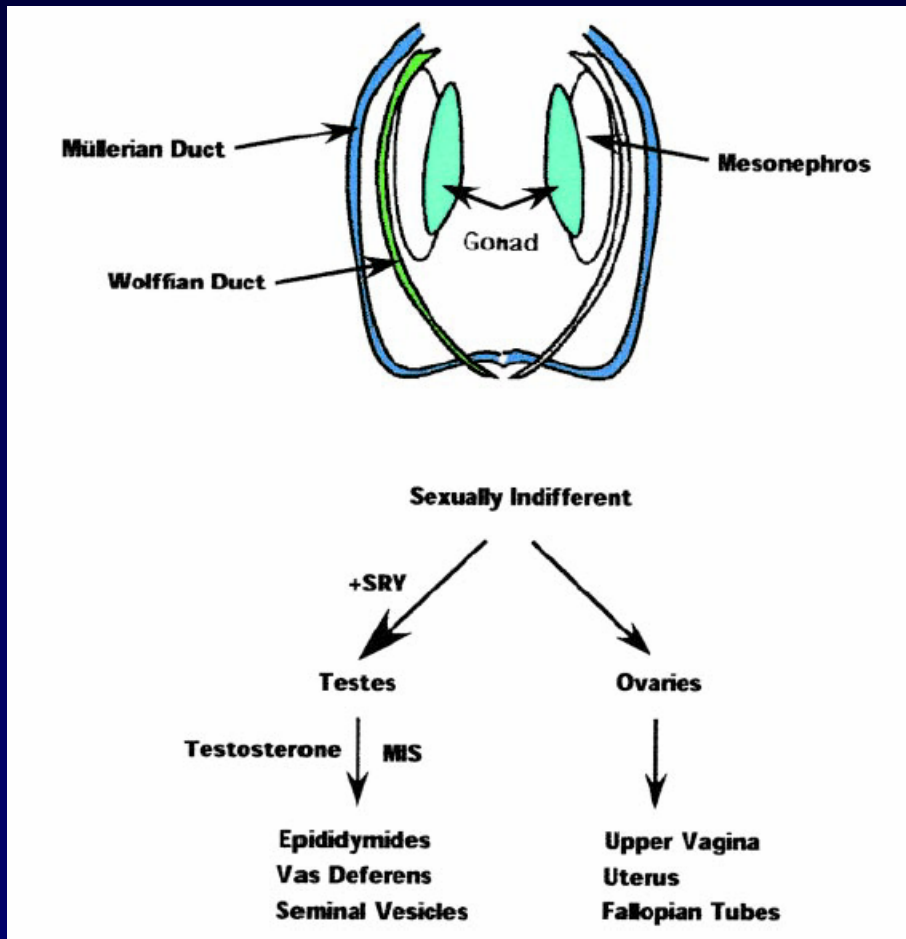
- **Typically diagnosed late (usually due to enlargement of the abdomen)**
- **Approximately 22,000 women were diagnosed with ovarian cancer in the U.S. in 2007**
- **Approximately 15,000 women died of this disease in 2007 in the U.S.**
- **Overall, the one-year survival rate is 76% and the five-year survival rate is 45%**
- **New therapeutic approaches are desperately needed**

We have targeted a functional ovarian cancer antigen with minimal normal tissue expression

Müllerian Inhibiting Substance Type II Receptor (MISIIR)

- **MISIIR is a transmembrane serine/threonine kinase**
- **In adults, its expression is limited to ovarian surface epithelium, Leydig cells of the testis, and at lower levels in breast, prostate and motor neurons.**

MISIIR's Role in the Regression of the Female Reproductive Tract



In the developing male the testes secrete MISIIR's ligand, the Mullerian Inhibiting Substance, (MIS) which binds to MISIIR expressed on the female reproductive tract

MISIIR Expression in Ovarian Cancer

- Typically tens of thousands of copies per cell
- Moderate or strong MISIIR expression is detected by Immunohistochemistry in a majority of gynecologic malignancies
 - Epithelial ovarian cancer (serous, clear cell, endometrioid mucinous)
 - 69% positive
 - Endometrial cancer
 - 75% positive

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

A) Benign Post-
Menopausal ovary
MISIIR “negative”

C) Serous Epithelial
Ovarian Cancer
1.25x “positive”

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

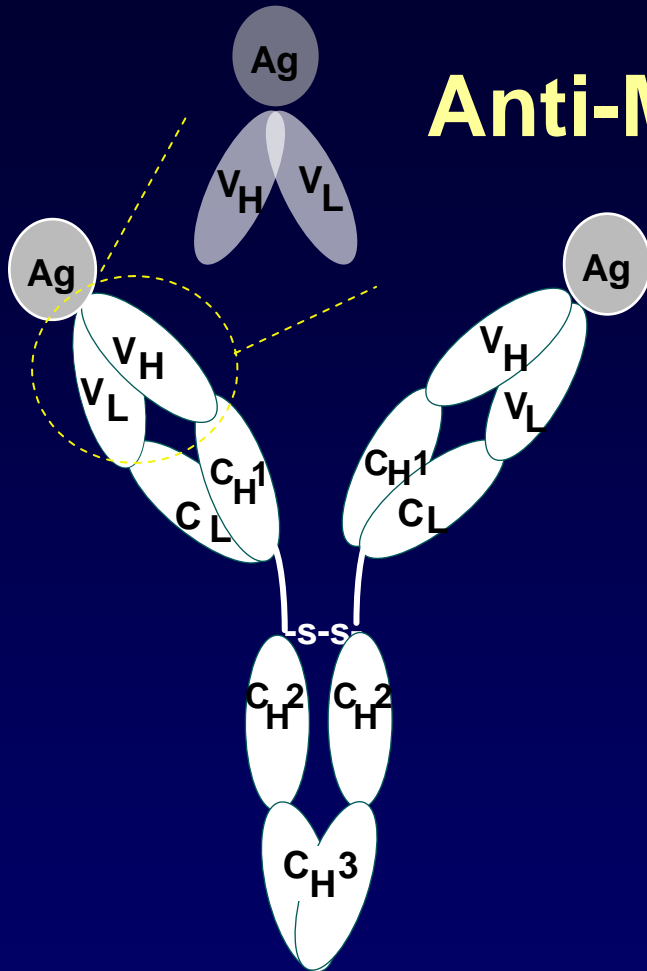
B) Post-Menopausal
OSE
MISIIR “negative”

D) Serous Epithelial
Ovarian Cancer
20x “positive”

MISIIR is a Relevant, Functional Target for the Treatment of Ovarian Cancer

- **Ovarian cancer cell lines undergo apoptosis following treatment with MIS in both *in vitro* and *in vivo* settings**
 - **MIS function is not restricted by species (human cells respond to MIS from a human, mouse, rat, etc)**
- **Primary ovarian cancer cells isolated from the ascities of ovarian cancer patients respond to MIS treatment**

Anti-MISIIR Antibodies



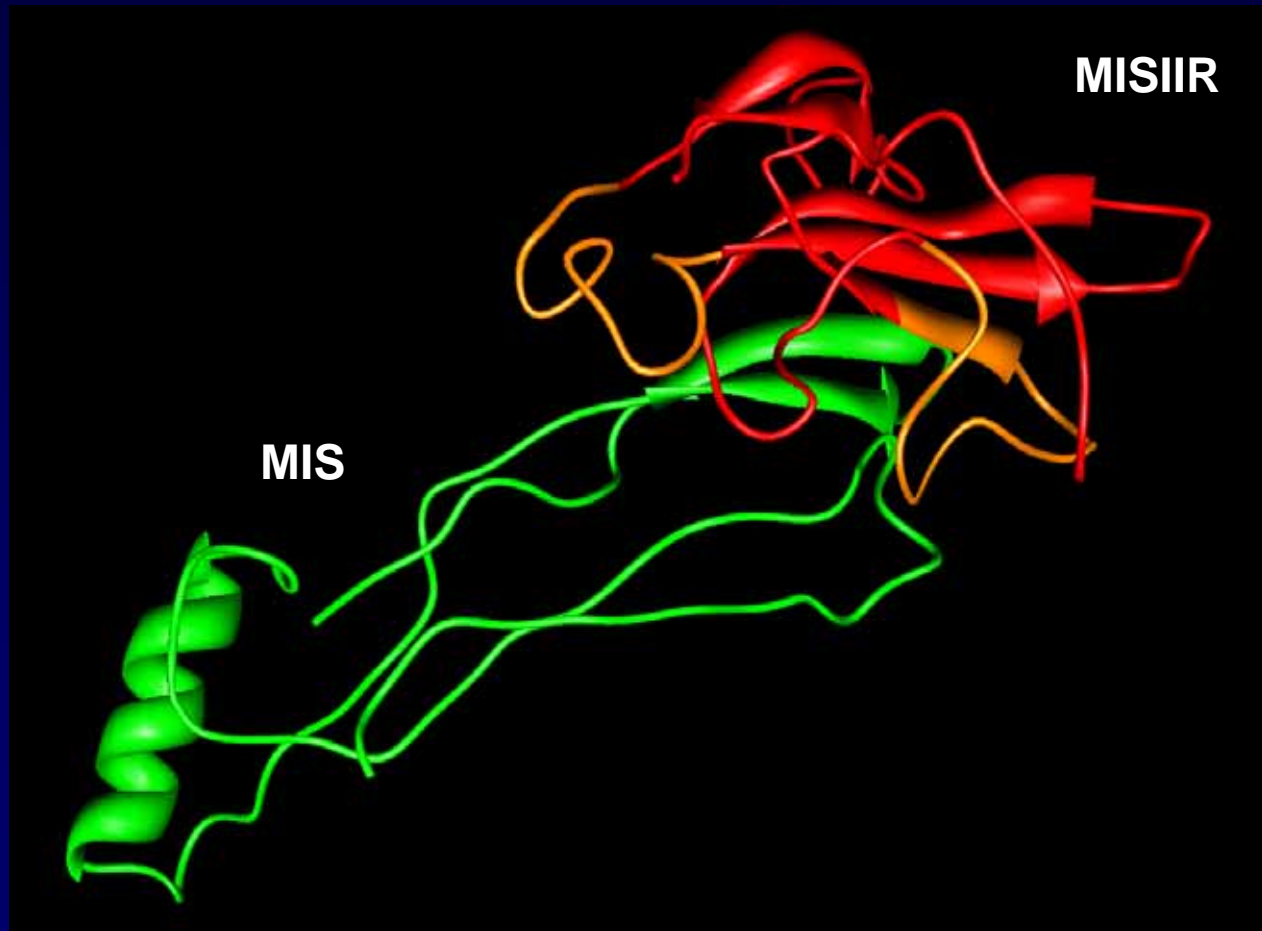
IgG
150 kDa

We cloned and expressed human MISIIR's extracellular domain

Using a number of techniques we have isolated anti-MISIIR single-chain Fv (scFv) molecules from two naïve human scFv phage display libraries

Modeled Potential MIS-MISIIR Contact Residues

Performed phage selections against potential
“critical peptides”

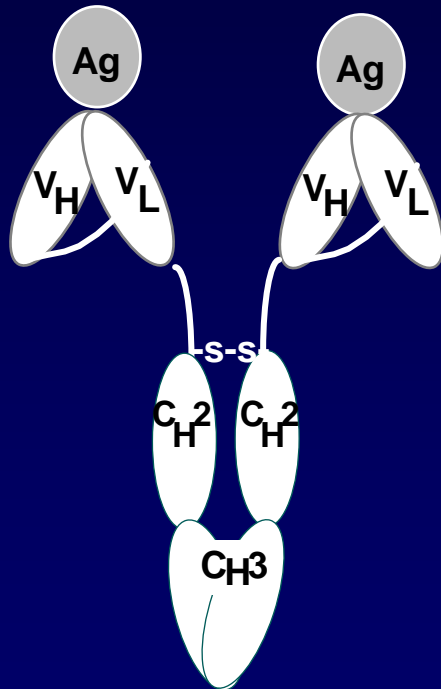


Using these techniques and classic hybridoma technology we have isolated over a dozen different antibodies that are specific for human MISIIR

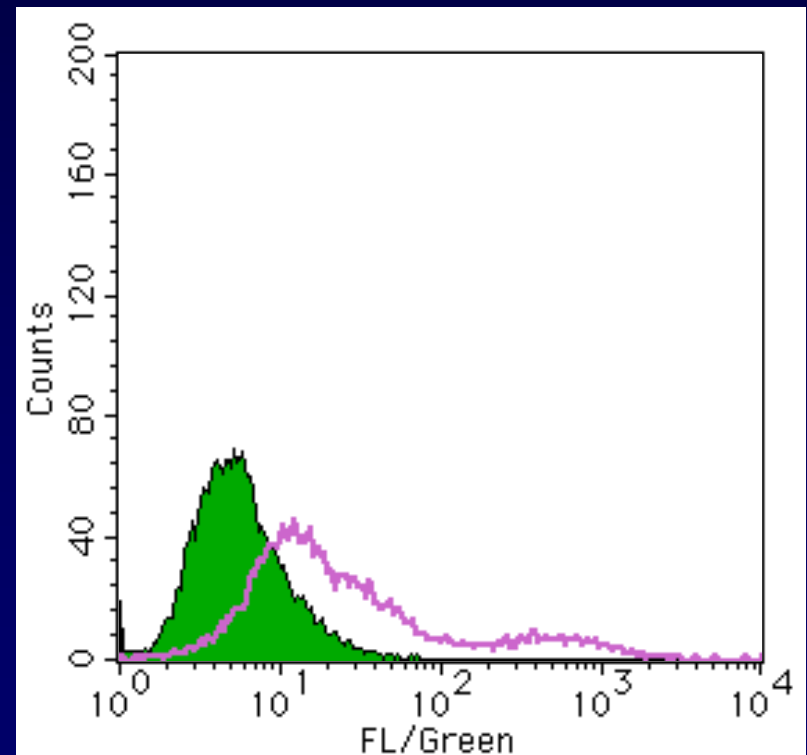
Lead Molecule: GY-4 scFv-Fc

We engineered an scFv dimer, GY-4 scFv-Fc, containing two anti-MISIIR scFv molecules and a human IgG1 Fc domain.

This molecule exhibits moderate binding to human AN3Ca endometrial cancer cells and mouse MOVCAR-1 cells by flow cytometry



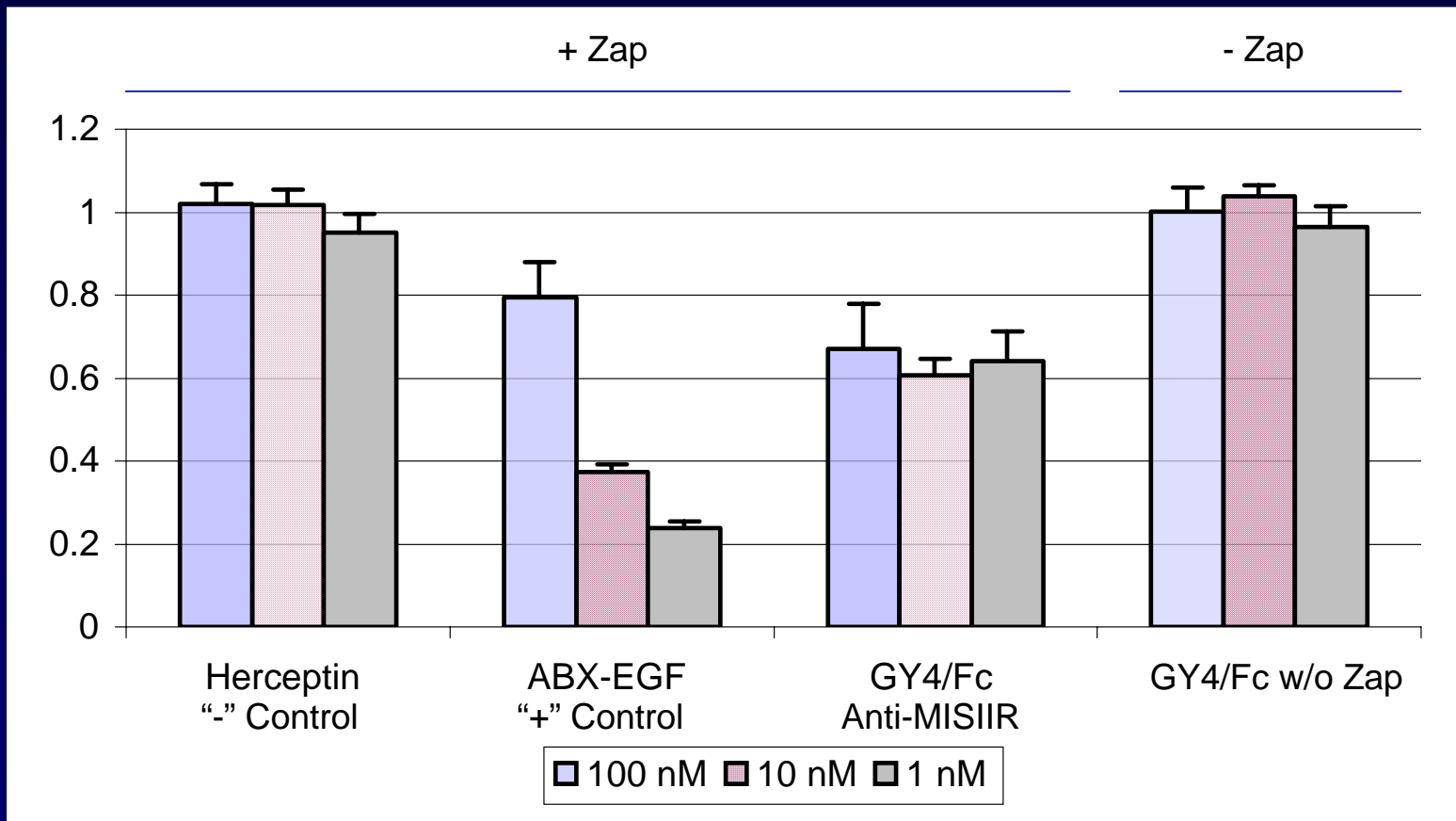
“GY-4”, an anti-MISIIR
scFv-Fc dimer



Binding to human AN3Ca endometrial
cancer cells

GY-4 scFv-Fc can Function as an Immunoconjugate

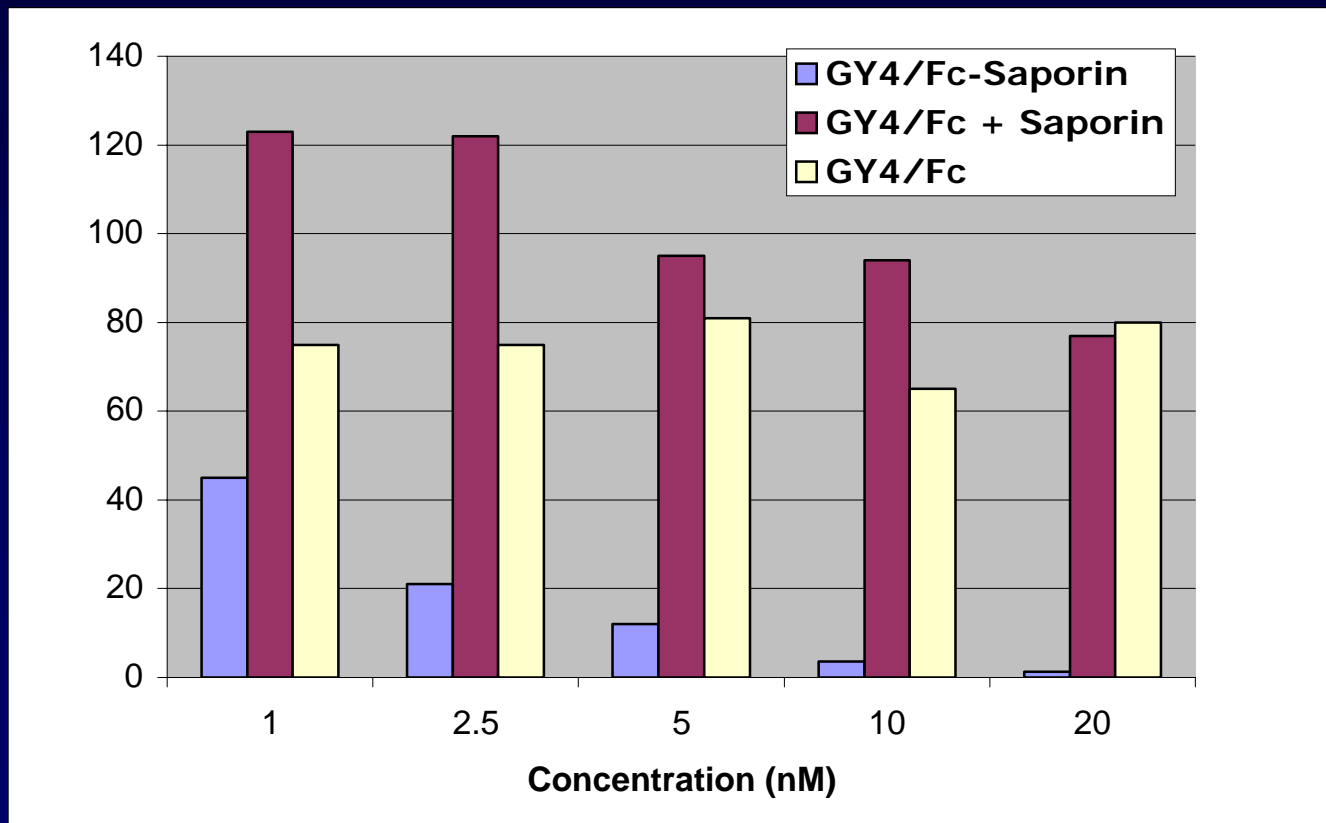
Potential as a carrier for cytotoxic agents was demonstrated using “ZAP”, a secondary anti-human IgG Fc MAb conjugated to the toxin saporin



MISIIR “+” human AN3Ca cells treated in a 96h MTT assay

GY-4 scFv-Fc-Saporin Conjugate

GY-4 scFv-Fc conjugated directly to saporin demonstrates dose dependent targeted killing of tumor cells expressing human or mouse MISIIR



MISIIR “+” Mouse MOVCAR1 cells treated in a 96h MTT assay

Conclusions

- **We have developed engineered “human” antibodies that target human MISIR, a highly relevant target for ovarian cancer therapy**
- **Our lead antibody, GY-4 scFv-Fc, is capable of selectively targeting a cytotoxic agent to MISIR positive human and mouse tumor cells**
 - **This rare cross-species reactivity allows us to perform potentially relevant efficacy and toxicity assays in the preclinical stage, thereby reducing the risks associated with clinical trials**

Current Efforts

- We are validating the efficacy and toxicity of anti-MISIIR immunoconjugates *in vivo*
- We are cloning the GY-4 scFv-Fc molecule into an intact human IgG1 format
- We are aggressively attempting to develop antibodies that directly induce signaling through MISIIR as they may directly trigger cytotoxic effects. This is done by screening hybridoma supernatants and new scFv clones isolated by phage display using a reporter gene system

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Facilities

Molecular Modeling
Hybridoma
Oligonucleotide Synthesis
Flow Cytometry
Laboratory Animal

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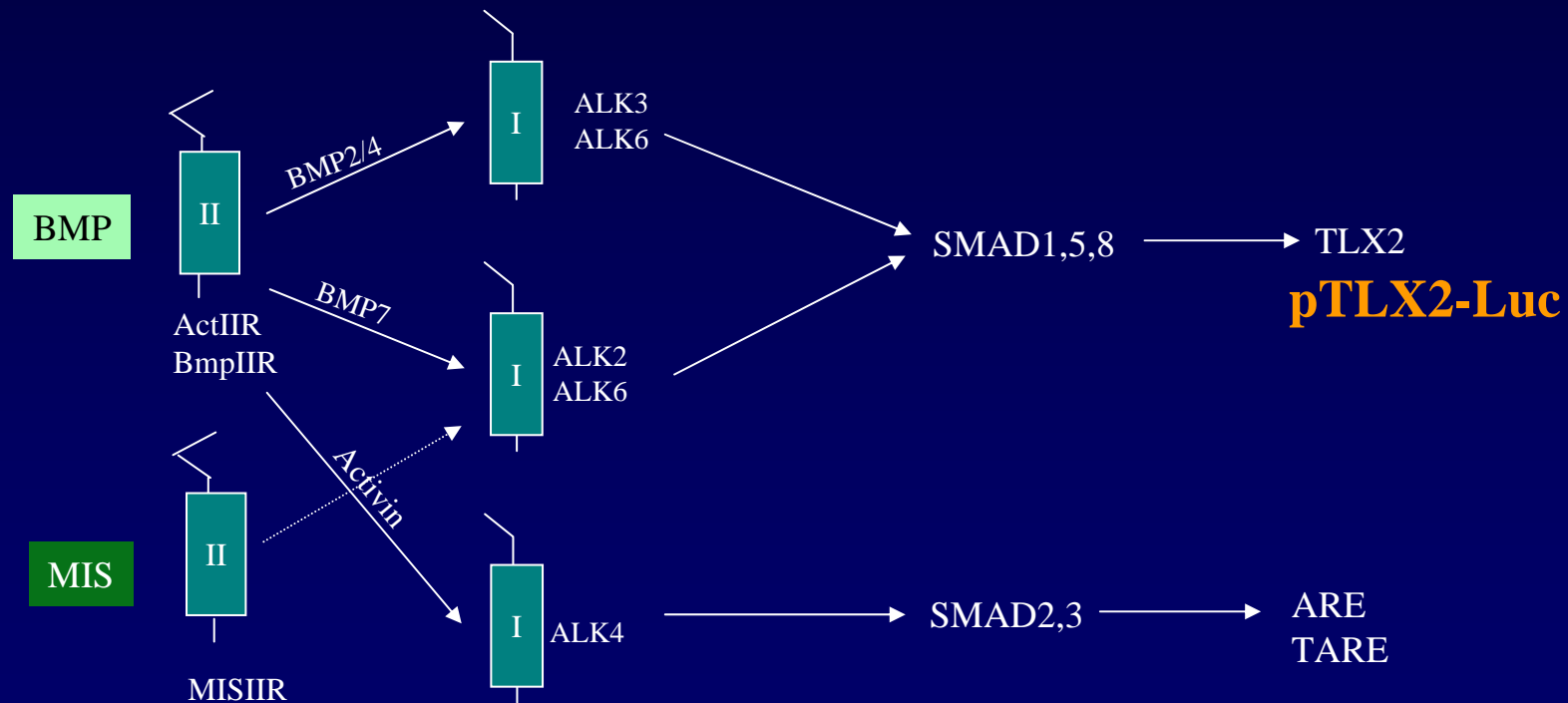
* Former lab member

TLX2

- The TLX-2 homeobox gene is a downstream target of BMP signalling and is required for mouse mesoderm development.
- Exogenous BMP-2 rapidly activates TLX-2 expression in the epiblast of E6.5 embryos.
- A TLX-2 promoter element responds to BMP-2, 4,7 signals in P19 cells, and this response is mediated by BMP type I receptors and Smad1.
- MIS activates a BMP-like signaling pathway, which is solely dependent on the presence of the MISR11 and bioactive MIS ligand.
- ALK2 is essential for MIS-induced signaling in two independent assays, the cellular TLX-2 reporter gene assay and the Mullerian duct regression organ culture assay.
- BMP dependent induction of BRE in the *TLX2* promoter is only observed in P19 mouse embryonic carcinoma ce11 line and in early embryos, indicating that ceU type specific nuclear factors may be required for such response. **No induction was detected in either HepG2, Mv1Lu, or MEFs**

Generation of a stable MISIIR Reporter Gene System

The *Tlx-2* (T-cell leukemia, homeobox2) gene is a downstream target of BMP and MIS signaling (Tang SJ et al. *Development* 125, 1877-1887 (1998)). Genes encoding mouse *TLX2*-Luciferase and human MISIIR were stably transfected into P19 mouse neuroblastoma cells that normally lack MISIIR.



Modified from Marina Macías-Silva, 1998

MISIIR Reporter Gene System Signals in Response to Both BMP2 and MIS

Both BMP2 and two forms of recombinant MIS (a gift of Dr. Donahoe, MGH) trigger signaling through our stable reporter gene system. (T= treatment, NT = no treatment)

