UPSTREAM PROCESS DEVELOPMENT FOR GBR 1302, A BISPECIFIC ANTIBODY BASED ON GLENMARK'S PROPRIETARY BEAT® (BISPECIFIC ENGAGMENT BY ANTIBODIES BASED ON T CELL RECEPTOR) FORMAT

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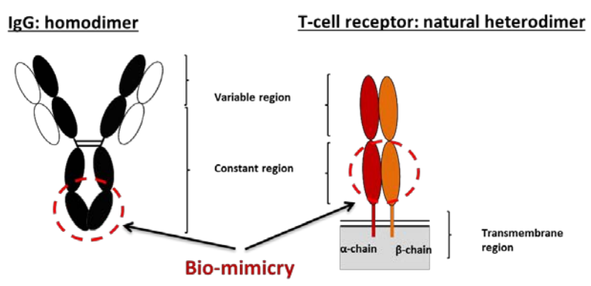
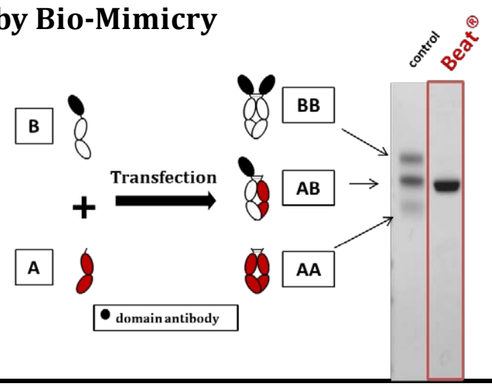
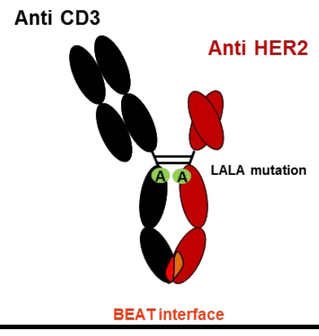
Glenmark Pharmaceutical's BEAT® platform is a novel bispecific heavy chain heterodimerization platform based on a unique concept of bio-mimicry. Using our BEAT® platform, we have developed a BEAT antibody –GBR 1302- designed to effectively recruit cytotoxic T cells against HER2 positive breast cancer cells including the trastuzumab-resistant breast cancer cell lines. GBR1302 is composed of three different subunits (HC, scFV-FC and LC) that are expressed in recombinant CHO-S cells. Herein, we describe the upstream process development which lead to an industrial scalable process.

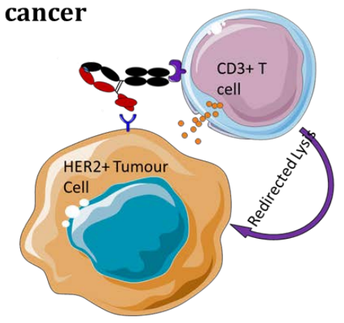
BEAT® Bispecific Antibody: Hc heterodimerization by Bio-Mimicry





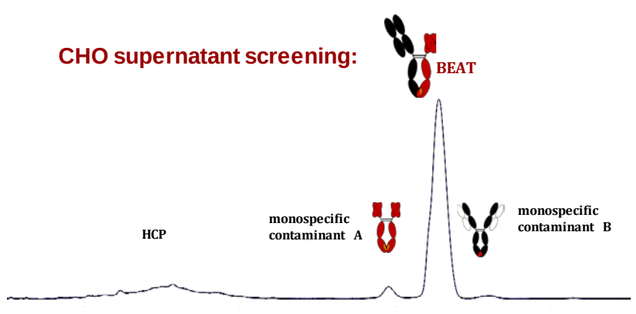
GBR 1302: first BEAT® in the clinic for HER2+ cancer

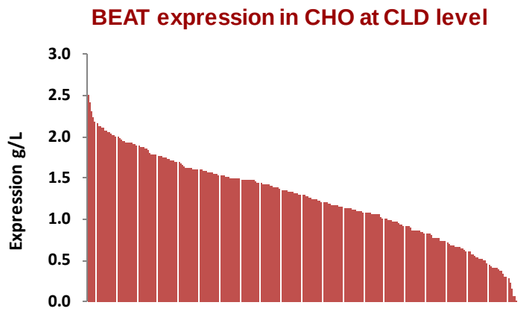


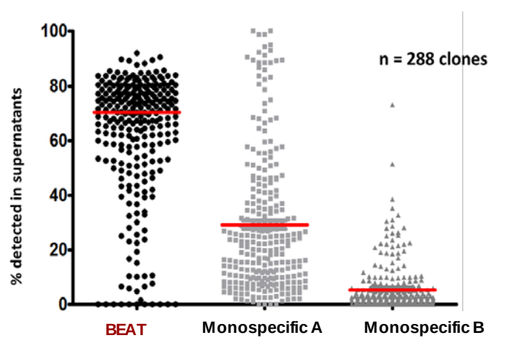


Mechanism of action of GBR 1302: Redirected lysis (RDL)

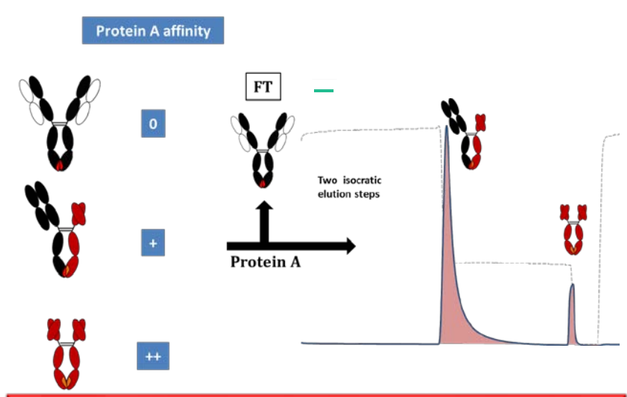
The BEAT® Platform in a nutshell:







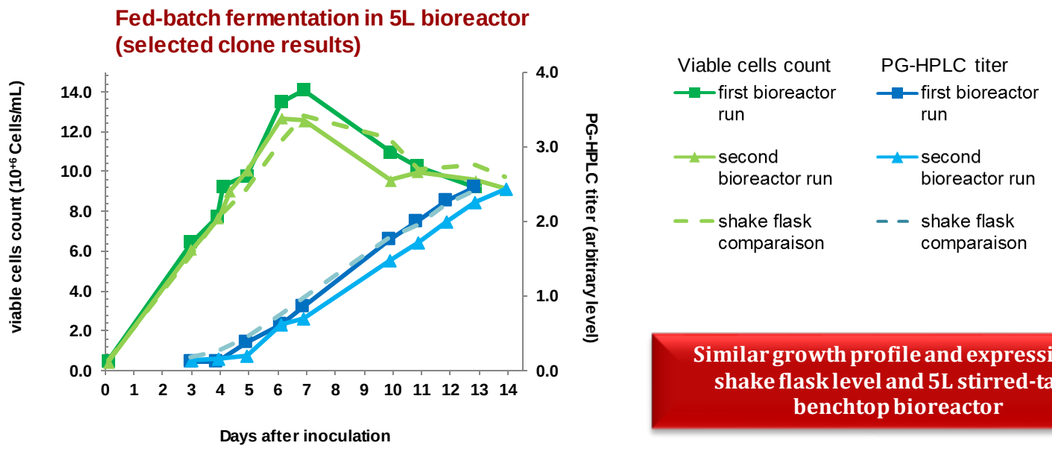
Up to 90 % bispecific product secreted



Built-in protA approach
Standard mAb purification process

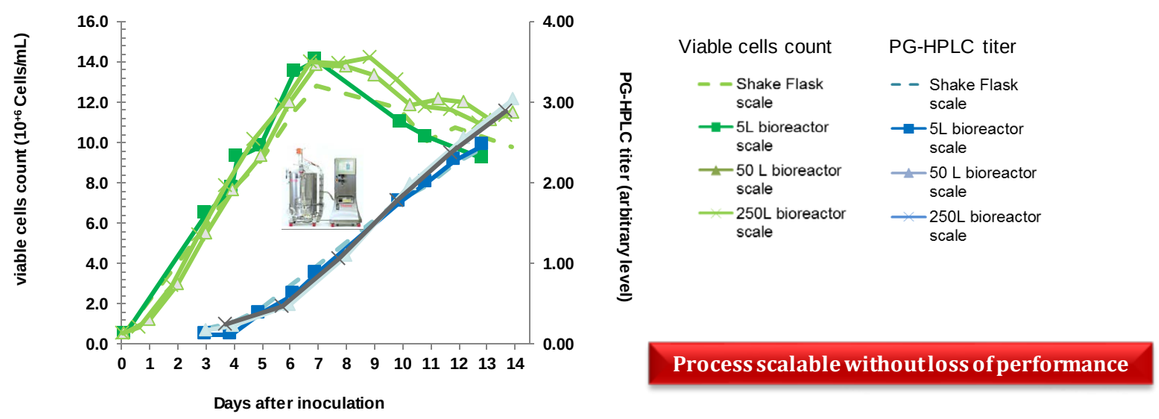
Intermediate scale (5L sSTB) development for GBR1302

- Media and feed selected based on small scale model
- Establishment of a controled process (gasing strategy optimization, pH, temperature)
- Duplicate runs



Similar growth profile and expression at shake flask level and 5L stirred-tank benchtop bioreactor

Scalable process from shake flask scale to 50L and 250L bioreactor (GMP)



Process scalable without loss of performance

USP process development for GBR1302

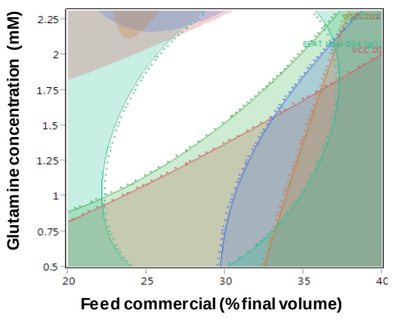
DOE based on media and feed optimization → 5 L intermediate scale → 50L / 250L (GMP) scales

Small-scale development for GBR1302

Model was build with the statistical software JMP 11.0

parameter estimates
Feed A+B (% final volume)
first feeding days
glutamine concentration (mM)
Feed A+B (% final volume) * Feed A+B (% final volume)
Feed A+B (% final volume) * first feeding days
Feed A+B (% final volume)* glutamine concentration (mM)
first feeding days * first feeding days
first feeding days * glutamine concentration (mM)
glutamine concentration (mM) * glutamine concentration (mM)

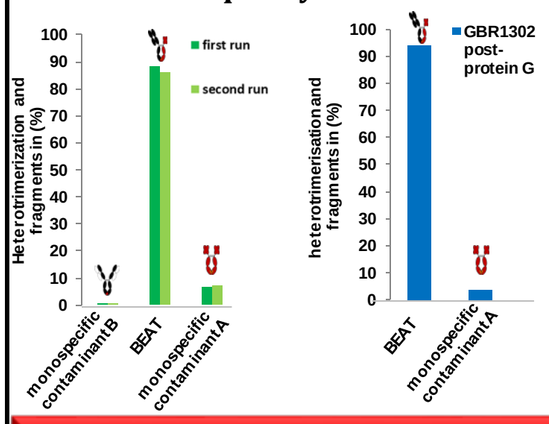
The figure below shows the response contours obtained for L-Glutamine and the feed quantitiy



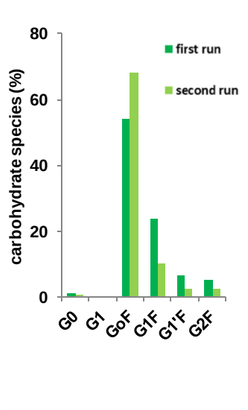
Response
VCC D7 (x 10^{+6} C/mL)
VCC D10 (x 10^{+6} C/mL)
V% D12 (%)
V% D14(%)
BEAT level D14 (g/L)

Selection of an optimal process for all candidates

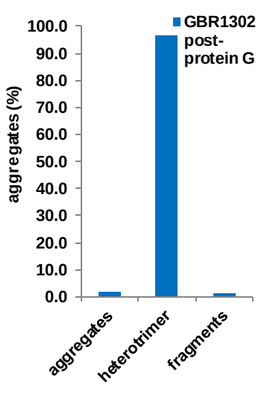
GBR1302 quality attributes



No impact of the process on heterotrimerization level
⇒ Up to 85% bispecific product at harvest
⇒ Up to 90% bispecific product after puirification



Standart CHO-S glycosylation profile



Low aggregate level (≤2%)

A standard Upstream process development lead to develop a feeding strategy and a 5L benchtop scale stirred-tank bioreactor process with similar titers to those determined during the Cell line development and the small-scale optimization runs. This process was successfully scaled up to 50L and 250L scale, without loss in performance.
The major product's quality attributes were not affected by the scale of production. The level of heterotrimerization, fragmentation, and aggregation remained within the same range. The glycosylation pattern of GBR 1302 was found to be within the range of that found with IgG antibodies produced using the same host cell.
These data show that the GBR 1302 BEAT antibody has characteristics in upstream process development that are very similar to a standard IgG antibody. The assembly of GBR 1302 is very robust and is not subject to modifications during the bioreactor process run at any scales.
The excellent manufacturing attributes of GBR1302 allows further clinical development as a treatment for HER2 positive cancers. Entry in phase I is expected in 2015.