

## Targeted Anticancer Therapeutics

### *Granzyme B and TNF*

Clayton Biotechnologies, Inc. is seeking corporate partners for the clinical development of targeted anticancer therapeutic proteins. Generated from a program conducted by the Clayton Foundation for Research under the scientific leadership of Dr. Michael Rosenblum, we offer a portfolio of product candidates based on our proprietary platform technologies. These platforms comprise highly cytotoxic proteins which can be chemically conjugated or genetically fused to targeting molecules such as antibodies, peptides, antibody-like scaffolds, cytokines, aptamers, and growth factors for the treatment of cancer, autoimmune diseases, proliferative eye diseases and other cell hyperproliferative disorders.

#### Product Candidates

Our pipeline of therapeutic conjugated immunotoxins includes a product that is currently in clinical testing, several advanced pre-clinical products and recombinant fusion constructs which are in various stages of R&D development. These products are linked to our proprietary payloads such as human tumor necrosis factor (TNF) and human granzyme B (GrB).

Compound Name	Target	Indications	Status
<b>GrB/scFvMel</b>	MAb scFv fragment (gp240) fusion protein	Melanoma, Brain and Lobular Breast Cancer	Animal model efficacy
<b>scFvMel/TNF-a</b>	MAb scFv fragment (gp240) fusion protein	Melanoma, brain and Lobular Breast Cancer	Advanced pre-clinical
<b>GrB/ML3-9</b>	HER2	Breast, colon, lung	express/characterize
<b>GrB/IIP45X5</b>	Solid Tumors	Brain, breast, melanoma	express/characterize

#### Platform Technology

Our products are developed out of our unique and proprietary platform technology focused on highly cytotoxic protein payloads which can be either chemically conjugated or genetically fused to virtually any cell-targeting molecule. Our R&D programs are focused around distinct platform technologies, each of which has a different mechanism of action: 1) human granzyme B(GrB) which is a highly cytotoxic mediator of the immune response, and 2) TNF, a normal human cytokine highly cytotoxic to cells through interaction with specific cell-surface receptors.

#### Intellectual Property

Clayton Biotechnologies has been aggressive in obtaining broad, world-wide intellectual property protection for key aspects of the Rosenblum targeting portfolio, with an emphasis on protection both for the payload GrB as well as, where appropriate, the targeting molecules.

#### Corporate Partnering

Several of our product candidates have been developed through collaborations with other academic institutions. We also have initiated collaborations with leading biotechnology companies in which we are coupling our payloads to their proprietary targeting molecules. Given our extensive experience with these payloads, we can assist our partners with advice on pre-clinical development strategies for clinical approaches with these constructs.

*Please contact our business development unit if you are interested in accessing, via licensing or collaboration, one of our platform technologies to combine with your targeting biopharmaceuticals. Several of our candidate products are also available for licensing.*

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## Platform Technology - Cytotoxic Protein Payloads

The Clayton Foundation for Research is conducting its *Targeted Anticancer Therapeutics Program* under the leadership of Dr. Michael Rosenblum, professor at MD Anderson and Dr. Stephen Howell, professor at University of California – San Diego. We have developed several broad platforms of proprietary, highly toxic cytotherapeutic proteins, which can be chemically conjugated or genetically fused to various targeting biopharmaceuticals.

Our therapeutic constructs overcome several major problems faced by developers of therapeutic antibodies and other tumor-targeting molecules. Naked antibodies and targeting molecules by themselves are often not potent enough to kill clinically meaningful amounts of tumor. While the potency of antibodies and targeting molecules can be enhanced by chemically conjugating or fusing them to conventional toxins, in most cases, this results in increased toxicity to normal tissues.

Our conjugated immunotoxins have very important advantages over conventional toxins for improving the therapeutic index of naked antibodies or other targeting moieties.

*These advantages include:*

- Improved potency and therapeutic index versus conventional toxins
- Lower systemic toxicity than conventional toxins due to their inherent inability to enter normal cells without a targeting molecule
- No need for complex linker technologies
- Can be used with non-antibody targeting molecules
- Some therapeutic payloads do not have to be endocytosed to kill cancer cells
- Novel Mechanism of Action-Synergistic with other Agents/Modalities
- The targeted therapeutic can potentially be used for imaging to select patients likely to respond to therapy

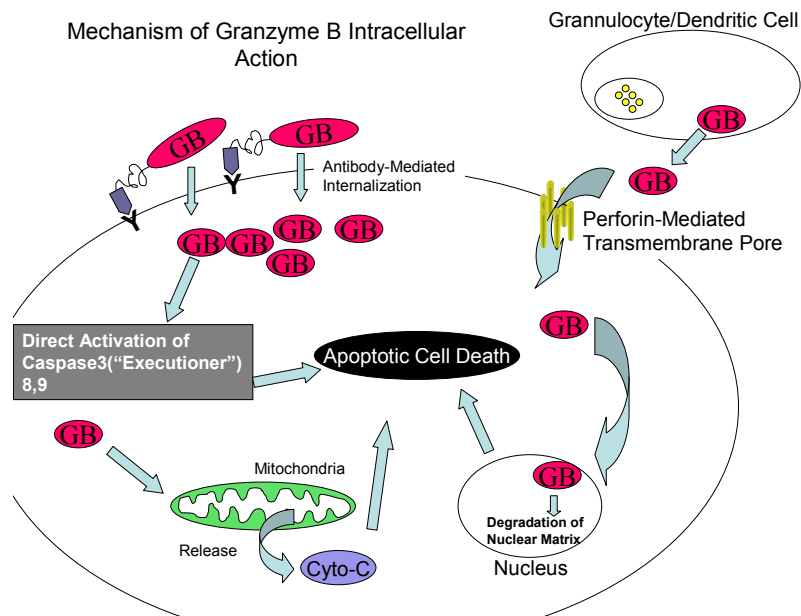
Dr. Rosenblum has developed distinct platform technologies with different mechanisms of action. Each of these payloads offer advantages unique to specific classes of targeting molecules and diseases. Multiple mechanisms of cytotoxic action provide additional protection against drug resistance.

Payload	Source	Mechanisms
<b>GrB - Granzyme B</b>	Human recombinant	Apoptosis Internalization required
<b>TNF-<math>\alpha</math> - Tumor Necrosis Factor-alpha</b>	Human recombinant	Receptor mediated-apoptosis No internalization required

## GrB - Granzyme B Payload

To destroy target cells, cytotoxic cells in the human immune system first release perforins to permeabilize the cell membrane of the cell being attacked. The serine protease granzyme B (GrB) is then released and enters the target cell through pores created by perforin. Granzyme B then generates an intense pro-apoptotic signal through direct cleavage of the effector caspases 3, 8 and 9. In addition, granzyme B works directly on mitochondria causing release of Cytochrome C. Thirdly, Granzyme B directly cleaves nuclear matrix material. The end result of these three different mechanisms is an intense, irreversible, pro-apoptotic effect that commits the cell to death.

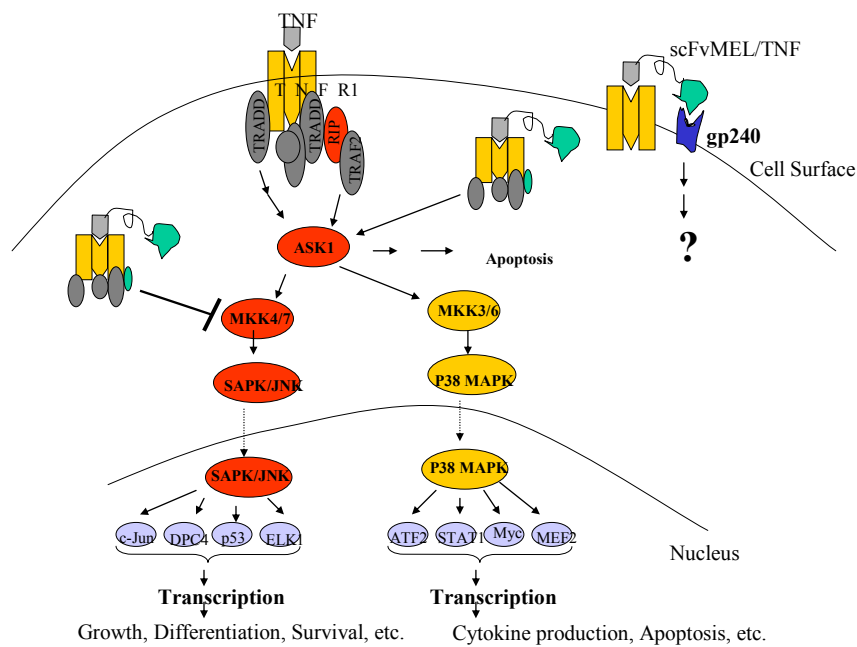
Granzyme B-based fusion constructs kill cancer cells in a perforin-independent manner using these three distinct cytotoxic mechanisms. Dr. Rosenblum has developed a series of unique fusion constructs in which mature human granzyme B has been fused to various targeting moieties. The targeting moieties and their target receptors mediate endocytosis without the need for perforins. Fusion constructs containing GrB are biologically active without release of the molecule from the targeting moiety. Therefore, as in the case of the other payloads, there is no need for a cleavable linker. Cell-targeted GrB can be identified in the cytoplasm of tumor cells *in vitro* within 1 hr of the start of exposure. Targeting GrB to tumor cells results in a rapid, intense cytotoxic effect rapidly after drug exposure. Efficient killing of tumor cells in culture by GrB-containing recombinant constructs is produced by nanomolar concentrations.



## TNF- $\alpha$ - Tumor Necrosis Factor-alpha Payload

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a 17 kDa human protein which exists in solution as a compact trimer. TNF- $\alpha$  is one of the primary cytotoxic proteins of the human immune system and its potent cell-killing effects are mediated through interaction with two cell-surface receptors. Studies in Dr. Rosenblum's lab and elsewhere have demonstrated convincingly that TNF- $\alpha$  conjugated to a targeting moiety can generate impressive cytotoxic effects both in vitro and in vivo even when the tumor cells are resistant to unconjugated TNF- $\alpha$  itself. In vivo studies in tumor xenograft models also demonstrate specific antibody-mediated cytotoxic effects. As a recombinant version of a naturally-occurring human protein, TNF- $\alpha$  is likely to exhibit low immunogenicity.

In contrast to both rGel and virtually all other known toxins used for pharmaceutical targeting, TNF- $\alpha$  does not have to be delivered to the interior of the tumor cell; it just needs to be concentrated by the targeting moiety at the surface of the cell where it can then bind to its cell-surface receptors and trigger its cytotoxic action. TNF- $\alpha$ , like rGel, can either be chemically conjugated or genetically fused to targeting moieties.



## Pre-clinical Candidate Product: scFvMEL/TNF- $\alpha$

**Indications:** Melanoma and other gp240+ tumors

**Stage:** Advanced Pre-clinical

**Overview:** scFvMEL/TNF- $\alpha$  is being developed for the treatment of metastatic melanoma and other gp240+ (High Molecular Weight Melanoma-Associated Antigen-positive) tumors.

ScFvMEL/TNF- $\alpha$  is an unglycosylated, low molecular weight (45 kDa) recombinant fusion protein consisting of a single chain antibody fragment (scFvMEL) that targets a high percentage of melanomas, genetically fused to the human cytokine tumor necrosis factor-alpha (TNF- $\alpha$ ). TNF-alpha itself naturally forms into a compact trimer in solution. Analysis of ScFvMEL/TNF- $\alpha$  also demonstrates that the fusion construct exists in solution as a trimer of ~135 kDa. ScFvMEL/TNF- $\alpha$  is manufactured in *E. coli*.

ScFvMEL consists of a small flexible linker engineered between the variable domains of the heavy and light chains of the antibody ZME-018 and attached via a tether (G4S) to TNF-alpha. This molecule has the binding properties of the original anti-melanoma antibody and contains biologically active cytokine.

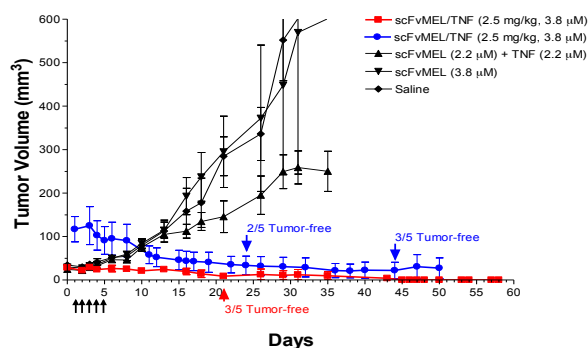
The progenitor ZME-018 antibody binds to a surface glycoprotein (gp240) found on over 80% of human melanoma cell lines and biopsy specimens. Studies have demonstrated that this antigen may be expressed on other tumor types, as well.

Pre-clinical studies on various human melanoma cell lines and melanoma (A-375) tumor bearing nude mice strongly indicate that scFvMEL/ TNF- $\alpha$  has potent cytotoxic activity both in vitro and in vivo and is an excellent candidate for clinical development. ScFvMEL/TNF- $\alpha$  also has a sister molecule, ScFvMEL/rGel, which uses recombinant gelonin instead of human TNF- $\alpha$  as its payload.

We have completed most preclinical development on scFvMEL/TNF- $\alpha$  including numerous efficacy and toxicity animal studies.

- Pre-IND package completed and submitted to FDA.
- Pre-IND guidance call held with FDA; agreement reached with the agency on clinical and pre-clinical requirements for Phase I initiation.
- Additional work required by FDA (<\$100K) initiated
- Phase I clinical protocol Approved by M.D. Anderson Cancer Center (MDACC) Institutional Review Board (IRB).
- MDACC Clinical Principal Investigator (PI) and team recruited and ready to proceed.
- Contract Manufacturing Organization (CMO) selected and inspected in detail.
- Manufacturing Process Instructions (MPI's) nearing completion.

Antitumor Activity of scFvMEL/TNF on A375GFP Xenograft tumors ( i. v. administration)



## R&D GrB candidate products

Compound Name	Target	Indications	Status
<b>GrB/scFvMel</b>	MAB scFv fragment (gp240) fusion protein	Melanoma, Brain and Lobular Breast Cancer	Animal model efficacy
<b>GrB/anti-CEA</b>	CEA	Solid Tumors	In vitro studies
<b>GrB/IIP45X5</b>	Solid Tumors	Brain, breast, melanoma	express/characterize
<b>GrB/ML3-9</b>	HER2	Breast, colon, lung	express/characterize
<b>GrB/BLyS</b>	BAFFR	Bcell, MCL, BCLL	express/characterize

Granzyme B is a human protein which is a highly cytotoxic mediator of the immune response. Also benefiting from excellent patent protection, we have developed a number of candidate products listed above through academic collaborations. Our studies have demonstrated the highly cytotoxic effect of granzyme B, which, at nanomolar concentrations, effectively kills tumor cells specifically targeted by the tumor cell targeting moiety. We are also focusing efforts on improving the production capabilities of Granzyme B.

## Other R&D candidate products

Compound Name	Target	Indications	Status
<b>scFvMEL/IkB</b>	gp240	Melanoma/Breast/Brain	In vitro and in vivo
<b>ML3-9/IkB</b>	HER2	breast, colon, lung	In vitro and in vivo
<b>ML3-9/TNF</b>	HER2	breast, colon, lung	In vitro and in vivo
<b>e-23/TNF</b>	HER2	breast, colon, lung	In vitro studies
<b>IkB/BLyS</b>	BAFFR	Bcell, MCL, BCLL	In vitro studies
<b>scFvMEL/BAX345</b>	gp240	Melanoma/Breast/Brain	In vitro studies

TNF (human tumor necrosis factor) is a normal human cytokine highly cytotoxic to cells through interaction with cell-surface receptors. The advantage of this cytotoxic payload is that it does not require cell internalization of the targeting molecule to confer cytotoxic activity.

## Patent Portfolio

Clayton Biotechnologies has been aggressive in obtaining broad, world-wide intellectual property protection for key aspects of the Rosenblum targeting portfolio, with an emphasis on protection both for the payloads (GrB) as well as, where appropriate, the targeting molecules.

Our granzyme B patent portfolio extends through 2022.

CLFR:012 TITLE: THERAPEUTIC AGENTS COMPRISING PRO-APOPTOTIC PROTEINS

Client Reference No.: NULL

Yuying LIU  
Michael G. ROSENBLUM

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	United States of America	Divisional	Allowed	12/788005	05/26/2010		
2	Australia	PCT National Phase	Issued	2002327310	07/17/2002	2002327310	01/11/2007
3	Canada	PCT National Phase	Issued	2454048	07/17/2002	2454048	05/03/2011
4	China	PCT National Phase	Pending	02817979.X	07/17/2002		
5	European Patent Office	PCT National Phase	Pending	02763328.8	07/17/2002		
6	Israel	PCT National Phase	Issued	159894	07/17/2002	159894	08/18/2010
7	Japan	PCT National Phase	Abandoned	2003-513498	07/17/2002		
8	Republic of Korea	PCT National Phase	Issued	10-2004-7000751	07/17/2002	10-891272	03/25/2009
9	New Zealand	PCT National Phase	Issued	530582	07/17/2002	530582	10/09/2008
10	Russian Federation	PCT National Phase	Issued	2004104369	07/17/2002	2319709	03/20/2008
11	Russian Federation	Standard	Never Filed				
12	United States of America	National	Issued	10/196793	07/17/2002	7101977	09/05/2006
13	United States of America	Divisional	Issued	11/415342	05/01/2006	7371723	05/13/2008
14	United States of America	Divisional	Issued	12/040111	02/29/2008	7759091	07/20/2010
15	United States of America	Provisional	Expired	60/306091	07/17/2001		
16	United States of America	Provisional	Expired	60/332886	11/06/2001		
17	United States of America	Provisional	Expired	60/360361	02/28/2002		
18	Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US2002/023378	07/17/2002		
19	South Africa	PCT National Phase	Issued	2004/0228	07/17/2002	2004/0228	04/26/2006

CLFR:239

TITLE: VASCULAR ENDOTHELIAL GROWTH FACTOR FUSION CONSTRUCTS USED TO INHIBIT OSTEOCLASTOGENESIS

**Summary:** Methods of treating osteoclastogenesis involving administering a conjugate comprising a cytotoxic molecule and a peptide that binds both vascular endothelial growth factor (VEGF) receptor type 1 (flt1) and VEGF receptor type 2 (kinase domain receptor/Flk-1). For example, VEGF-gelonin and VEGF-granzyme B fusion proteins. (FF)

Client Reference No.: AO-D-6519-12-3

Michael G. ROSENBLUM

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	United States of America	Continuation in Part	Issued	10/919193	08/16/2004	7601341	10/13/2009
2	United States of America	Continuation	Published	12/552103	09/01/2009		
3	Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US2005/029057	08/16/2005		