

Targeted Anticancer Therapeutics

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, diabetic retinopathy 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 gelonin (rGel), designer gelonins (drGel), human tumor necrosis factor(TNF) and human granzyme (GrB). *A more extensive list is provided on pages 6-7.*

Compound Name	Target	Indications	Status
GrB/scFvMel	MAb scFv fragment (gp240) fusion protein	Melanoma, Brain and Lobular Breast Cancer	Animal model efficacy
scFvMel/TNF-a	MAb scFv fragment (gp240) fusion protein	Melanoma, brain and Lobular Breast Cancer	IND complete <i>See pp 17-19</i>
HuM195/rGel	Antibody (anti-CD33) chemical conjugate	AML, CML Myelodysplastic Syndrome	Phase I (2 studies)

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 three distinct platform technologies, each of which has a different mechanism of action: 1) recombinant gelonin(rGel) which is a highly cytotoxic toxin originally derived from plants, and designer gelonin(drGel), which was developed to overcome concerns regarding immunogenicity associated with a non-human protein, 2) human granzyme B(GrB) which is a highly cytotoxic mediator of the immune response, and 3) TNF, a normal human cytokine highly cytotoxic to cells through interaction with specific cell-surface receptors *See pages 2-5.*

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 payloads (rGel, drGel and GrB) as well as, where appropriate, the targeting molecules. Please refer to *pages 8-16* of this brochure for a listing of exemplary patents and applications, demonstrating protection for drGel and GrB extending into the 2020s.

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 actively assist our partners with advice on pre-clinical development strategies as well as providing advice on 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, world-renowned professor at MD Anderson. 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

When a fusion product is desired, it can frequently be manufactured as a single protein in *E. coli* which offers the advantages of: inexpensive one-step bacterial fermentation; no need for a chemical conjugation step; ease of manufacture; potentially lower cost of goods sold.

Dr. Rosenblum has developed distinct platform technologies with three 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.

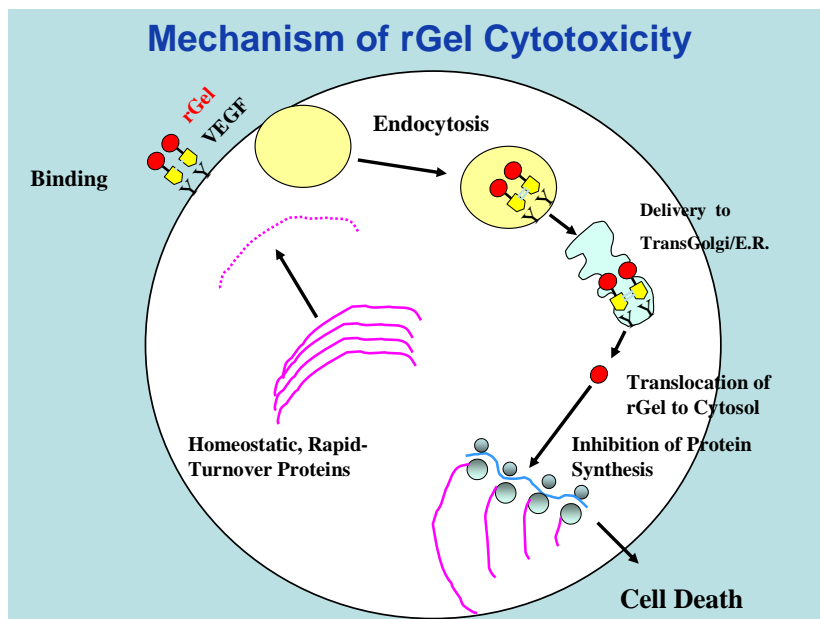
Payload	Source	Mechanisms
rGel and drGel Recombinant & Designer Gelonin	Plant (Recombinant, non-glycosylated) Designer gelonins are de-immunized	Ribosomal Inhibition Internalization is required
GrB - Granzyme B	Human recombinant	Apoptosis Internalization required
TNF-α - Tumor Necrosis Factor-alpha	Human recombinant	Receptor mediated-apoptosis No internalization required

Platform Technology

rGel & drGel- Gelonin Payloads

Gelonin is a small (28 kDa) protein that is extremely toxic when it gets inside cells, but cannot get into cells in significant concentrations unless it is coupled to a targeting moiety. This gives gelonin very low systemic toxicity alone, or when used in conjunction with many targeting moieties.

Gelonin is an N-glycosidase that cleaves adenine from mammalian ribosomal RNA thereby ablating protein synthesis and resulting in cell death. A recombinant, de-glycosylated version of gelonin (rGel) has been expressed in bacteria and is biologically equi-potent to the natural substance. As with many toxins of this class, a single molecule of rGel delivered to the cytoplasmic compartment is sufficient to irreversibly intoxicate a target cell. By itself, rGel does not bind to or become internalized into cells and thus has very little toxicity unless it is conjugated or fused to a targeting moiety. rGel can be chemically conjugated or genetically fused to many targeting moieties, such as antibodies or growth factors, that become internalized into the cells to which they bind. The original sequence of the gelonin molecule has been modified to include a free cysteine in an exposed external loop so that it can be chemically conjugated to targeting moieties through a site-specific disulfide linkage. This insures that no inactivation of the toxin molecule occurs during conjugation, and that the rGel remains tightly attached to the targeting moiety while it is in the bloodstream.



rGel can also be genetically fused at either its N- or C-terminal end to targeting moieties. Like our other payloads, gelonin retains full biologic activity when fused in this manner. This gives them a unique flexibility and utility versus other payload technologies.

rGel fusion constructs and chemical conjugates are cytotoxic to targeted cells at nanomolar concentrations. Conjugation or fusion of rGel to a targeting moiety increases its cytotoxicity by a factor of 100-1,000. rGel-containing conjugates and fusion constructs have plasma clearance and tissue distribution properties similar to those of the targeting moiety itself, in in vivo animal models.

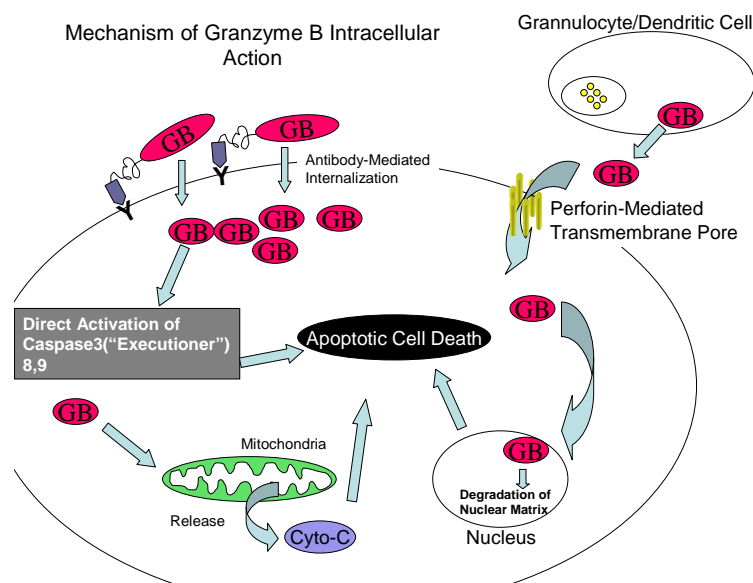
Numerous pre-clinical studies have been conducted with gelonin-based chemical conjugates and fusion constructs, and one product (HuM195/rGel) is currently in the clinic. Two human clinical trials of an rGel product that targets human leukemia cells are under way at M. D. Anderson Cancer Center.

Our designer gelonin payloads have the same mechanism of action as other Ribosomal Inhibitory Peptides (RIPs) such as gelonin, ricin and others, but have been genetically engineered to improve certain characteristics. For example, wherever possible, potentially antigenic sites not essential to cytotoxic function have been removed. Other plant-derived portions of the molecules, such as nucleic acid binding sites, have been replaced with human homologs. These changes have resulted in smaller, more compact molecules (e.g. ~19 kDa) that are thought to minimize potential antigenicity. Nevertheless, gelonin has, to date, exhibited only minimal antigenicity in human clinical trials.

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, 7 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.

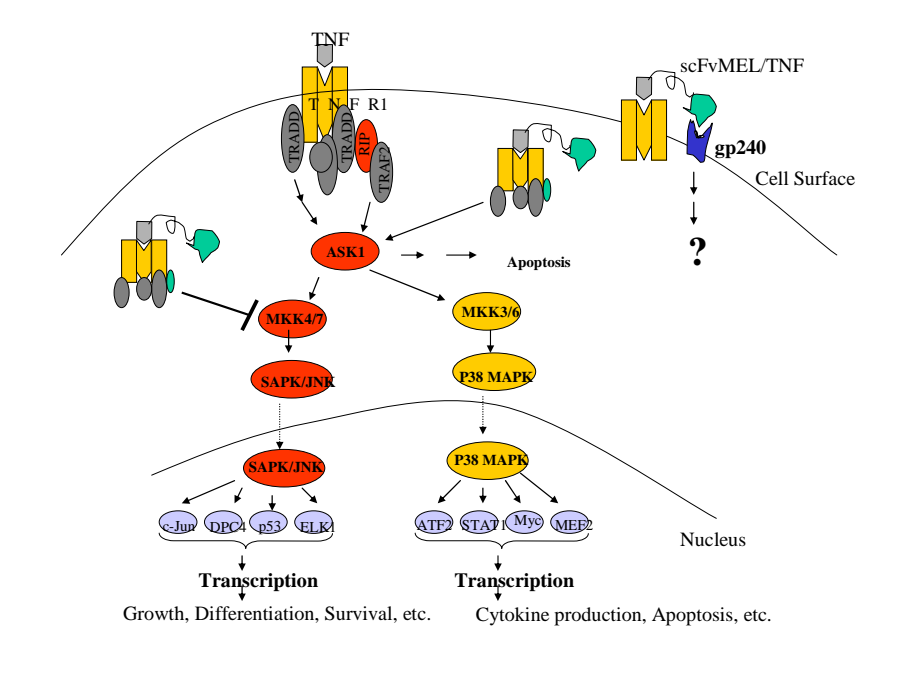
Granzyme B-based fusion constructs kill cancer cells in a perforin-independent manner using these three distinct cytotoxic mechanisms. Dr. Rosenblum has developed a series of unique fusion constructs in which mature human granzyme B has been fused to various targeting moieties. The targeting moieties 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- α - Tumor Necrosis Factor-alpha Payload

Tumor necrosis factor-alpha (TNF- α) is a 17 kDa human protein which exists in solution as a compact trimer. TNF- α 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- α 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- α 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- α is likely to exhibit low immunogenicity.

In contrast to both rGel and virtually all other known toxins used for pharmaceutical targeting, TNF- α 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- α , like rGel, can either be chemically conjugated or genetically fused to targeting moieties.



Products Currently in Development

Clinical and Preclinical stage candidate products

Compound Name	Target	Indications	Status
HuM195/rGel	Antibody (anti-CD33) chemical conjugate	AML, CML Myelodysplastic Syndrome	Phase I (2 studies)
scFvMel/TNF-α	MAB scFv fragment (gp240) fusion protein	Melanoma, brain and Lobular Breast Cancer	IND complete <i>See pages 18-19</i>
scFvMel/rGel	MAB scFv fragment (gp240) fusion protein	Melanoma, brain and Lobular Breast Cancer	Tox, PK and Pre-IND <i>See page 17</i>
rGel/BLyS	BAFFR	B cell, MCL, BCLL	Tox, PK and Pre-IND

HuM195/rGel, rGel is chemically conjugated to a recombinant, humanized monoclonal antibody (HuM195) directed to the CD33 antigen that is expressed on the malignant cells in most patients with myelogenous leukemias. The naked HuM195 antibody was developed by Protein Design Labs and is now in clinical development by Seattle Genetics. Phase I studies on HuM195/rGel have demonstrated the safety of gelonin in humans. Immunogenicity of the complex appears to be low as only 2 of 21 evaluable patients developed detectable Human Anti-Gelonin Antibodies (HAGA). Nevertheless, we have actively addressed the issue of immunogenicity by developing the designer gelonin platform, which is de-immunized gelonin.

scFvMel/rGel and scFvMEL/TNF fusion constructs are close to entering Phase I clinical trials. ScFvMEL/TNF is an immunocytokine for the specific delivery of TNF to melanoma and breast tumors. Pre-IND studies demonstrate that this molecule is extremely cytotoxic to cells specifically targeted by the scFvMEL antibody. In addition, this construct localizes specifically in tumor xenografts demonstrating its high level specificity.

R&D GrB candidate products

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

R&D rGel and drGel candidate products

Compound Name	Target	Indications	Status
ML3-9/rGel	HER2	Breast, colon, lung	In vitro and in vivo
Anti-CEA/rGel	CEA	Solid tumors	In vitro studies
HCG/rGel	LH receptor	Ovarian	In vitro studies
rGel/IIP45X5	Solid Tumors	Brain, breast, melanoma	Express/characterize
Anti-FGFr3/rGel	FGFR3	Bladder & multiple myeloma	Express/characterize

Gelonin is a highly cytotoxic ribosome inactivating protein originally derived from plants. Gelonin is chemically or recombinantly fused to a cell-targeting molecule. Designer gelonin is a de-immunized form of gelonin which benefits from extended patent coverage. Both forms of gelonin also have the advantage of ease of manufacturing.

While obtaining human clinical validation of gelonin, we are actively developing a pipeline of candidate products in partnership with academic institutions and commercial entities. In each case, our partner brings a unique cell-targeting molecule. These molecules have the property of effectively targeting cancer cells and allowing internalization, but require the cytotoxic activity of gelonin in order to become effective anticancer therapeutic agents.

Other R&D candidate products

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

CLFR:012

TITLE: THERAPEUTIC AGENTS COMPRISING PRO-APOPTOTIC PROTEINS

Summary: Chimeric polypeptides with cell targeting moieties and signal transduction factors. For example fusion proteins between antibody targeting molecules and apoptosis inducing factors, such as granzyme B and pro-apoptotic Bcl-2 family members. These chimeric molecules may be used in the treatment of cell proliferative diseases such as cancer.

Client Reference No.: CLFR:012

Yuying LIU
ROSENBLUM, MICHAEL G.

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	Australia	Standard via PCT	Issued	2002327310	07/17/2002	2002327310	01/11/2007
2	Canada	Standard via PCT	Pending	2454048	07/17/2002		
3	China	Standard via PCT	Pending	02817979.X	07/17/2002		
4	European Patent Office	Standard via PCT	Pending	02763328.8	07/17/2002		
5	Israel	Standard via PCT	Pending	159894	07/17/2002		
6	Japan	Standard via PCT	Pending	2003-513498	07/17/2002		
7	Republic of Korea	Standard via PCT	Pending	10-2004-7000751	07/17/2002		
8	New Zealand	Standard via PCT	Issued	530582	07/17/2002	530582	10/09/2008
9	Russian Federation	Standard via PCT	Issued	2004104369	07/17/2002	2319709	03/20/2008
10	Russian Federation	Standard	Never filed				
11	United States of America	National	Issued	10/196793	07/17/2002	7101977	09/05/2006
12	United States of America	Divisional	Issued	11/415342	05/01/2006	7371723	05/13/2008
13	United States of America	Divisional	Application Filed	12/040111	02/29/2008		
14	United States of America	Provisional	Expired	60/306,091	07/17/2001		
15	United States of America	Provisional	Expired	60/332,886	11/06/2001		
16	United States of America	Provisional	Expired	60/360,361	02/28/2002		
17	Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US2002/023378	07/17/2002		
18	South Africa	Standard via PCT	Issued	2004/0228	07/17/2002	2004/0228	04/26/2006

CLFR:057

TITLE: IMMUNOTOXINS DIRECTED AGAINST C-ERB-2 (HER-2/NEU) RELATED SURFACE ANTIGENS

Summary: An immunotoxin for killing tumor cells (human mammary carcinomas, ovarian carcinomas, lung carcinomas, etc.) that over-express the c-erbB-2 protein. The immunotoxin includes a protein, such as the Tab 250 or BACh-250 antibodies, and a plant derived toxin such as gelonin and its analogues.

Client Reference No.: D5425CIP

Laura K. Shawyer
Michael G. Rosenblum

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	United States of America	Divisional	Pending	10/964195	10/13/2004		

CLFR:059

TITLE: BLYS FUSION PROTEINS FOR TARGETING BLYS RECEPTOR AND METHODS FOR TREATMENT OF B-CELL PROLIFERATIVE DISORDERS

Summary: Delivery of targeted BLYS polypeptides conjugated to a cytotoxic agent for treating, preventing, and/or monitoring therapy for a B-cell proliferative disorder. The cytotoxic agent may comprise at least part of rGelonin.

Client Reference No.: NULL

Mi-Ae LYU
Lawrence CHEUNG
Michael G. ROSENBLUM

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	Australia	Standard via PCT	Application Filed	2006210769	02/01/2006		
2	Canada	Standard via PCT	Application Filed	2595904	02/01/2006		
3	China	Standard via PCT	Published	200680003470.7	02/01/2006		
4	European Patent Office	Standard via PCT	Published	06734163.6	02/01/2006		
5	India	Standard via PCT	Application Filed	5566/DELNP/2007	02/01/2006		
6	Japan	Standard via PCT	Published	2007-553376	02/01/2006		
7	Russian Federation	Standard via PCT	Application Filed	2007132875	02/01/2006		
8	Singapore	Standard via PCT	Application Filed	200705145-1	02/01/2006		
9	United States of America	National	Pending	11/345661	02/01/2006		
10	United States of America	Divisional	New case				
11	United States of America	Provisional	Expired	60/649,478	02/01/2005		
12	Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US2006/003547	02/01/2006		
13	South Africa	Standard via PCT	Application allowed	2007/05803	02/01/2006		

CLFR:065

TITLE: IMMUNOCONJUGATES FOR CANCER DIAGNOSIS AND THERAPY

Summary: Treatment of cancers such as breast cancer, cervical carcinoma, human breast carcinoma and the like. Administered as a therapeutic is immunotoxin comprising the 15A8 antibody and a toxin such as a cytotoxic drug, a biological response modifier or a detectable label. Gelonin coupled to the 15A8 antibody is particularly beneficial.

Client Reference No.: AO-D-4906-12-LSE-1

Michael G. ROSENBLUM
Renato Dulbecco
W. Ross Allen

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
	United States of America	Continuation	Issued	08/201134	09/24/1993	6669938	12/30/2003

CLFR:239

TITLE: VASCULAR ENDOTHELIAL GROWTH FACTOR-GELONIN FUSION CONSTRUCTS AND USES THEREOF

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	Application Filed	10/919193	08/16/2004		

CLFR:251

**TITLE: VASCULAR TARGETING OF OCULAR
NEOVASCULARIZATION**

Summary: Methods and compositions for treating eye diseases with polypeptide fusion molecules. Fusion molecules deliver toxins, pro-apoptotic sequences and/or anti-angiogenic sequences to cells via a VEGF targeting moiety.

Client Reference No.: NULL

Michael ROSENBLUM
Peter A. CAMPOCHIARO

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	Canada	Standard via PCT	Application Filed	2606989	04/28/2006		
2	European Patent Office	Standard via PCT	Published	06751936.3	04/28/2006		
3	Japan	Standard via PCT	Published	2008-509219	04/28/2006		
4	United States of America	National	Published	11/414782	04/28/2006		
5	United States of America	Provisional	Expired	60/675,958	04/29/2005		
6	Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US2006/016496	04/28/2006		

CLFR:314

**TITLE: MULTIMODALITY MOLECULAR IMAGING WITH
VASCULATURE-TARGETING FUSION TOXIN VEGF 121/rGEL**

Summary: scFvMEL/TNF VEGF/rGel constructs conjugated to Cu64-DOTA for imaging and personalization of therapies.

Client Reference No.: NULL

Xiaoyuan CHEN
ROSENBLUM, MICHAEL G.

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	United States of America	Provisional	Application Filed				

CLFR:269

**TITLE: CELL-TARGETED IKB AND METHODS FOR THE USE
THEREOF**

Summary: Activation of nuclear factor kB (NF-kB) is involved in a number of diseases such as viral and bacterial infections, and cell proliferative disorders such as cancer and autoimmune disease. In certain instances, constitutive NF-kB activity has also been linked to the resistance of certain cancers to chemo and radiation therapy. The instant invention concerns method of inhibiting NF-kB activity in target cell populations by delivery of a polypeptide inhibitor of NF-kB (IkB). Methods of the invention may be used to treat diseases such as infections, and cell proliferative disorders. Methods for sensitizing cells to apoptosis and cytotoxic therapies are also described.

Client Reference No.: NULL

LIU, YUYING
Michael G. ROSENBLUM

	Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1	United States of America	National	Published	11/679630	02/27/2007		
2	United States of America	Provisional	Expired	60/777,016	02/27/2006		
3	Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US2007/062887	02/27/2007		

CLFR:263

TITLE: IMMUNOTOXINS COMPRISING GELONIN AND AN ANTIBODY

Summary: Type I ribosome-inactivating proteins, such as gelonin, that have been modified for conjugation to additional molecules. For example, the ribosome inactivating protein may have a cysteine that can be used in forming a disulfide bond. These proteins may further be used for the targeted destruction of cells, for example in the treatment of cancer and autoimmune diseases.

Client Reference No.: NULL

Gary M. STUDNICKA
Marc D. BETTER
Stephen F. CARROLL

Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1 Canada	Standard via PCT	Issued	2162689	05/12/1994	2162689	07/18/2000
2 Switzerland	EP National Stage	Issued	94917380.1	05/12/1994	700,444	04/02/2003
3 Germany	EP National Stage	Issued	94917380.1	05/12/1994	0700444	04/02/2003
4 France	EP National Stage	Issued	94917380.1	05/12/1994	700,444	04/02/2003
5 United Kingdom	EP National Stage	Issued	94917380.1	05/12/1994	700,444	04/02/2003
6 Ireland	EP National Stage	Issued	94917380.1	05/12/1994	700,444	04/02/2003
7 European Patent Office	Standard via PCT	Expired	94917380.1	05/12/1994	EP700,444	04/02/2003
8 France	Standard via PCT	Never filed		05/12/1994		
9 Japan	Standard via PCT	Abandoned	6-525727	05/12/1994		
10 United States of America	Continuation in Part	Lapsed by inaction	08/064,691	05/12/1993		
11 United States of America	Continuation	Issued	08/425336	04/18/1995	5621083	04/15/1997
12 United States of America	Continuation	Issued	08/488113	06/07/1995	5744580	04/28/1998
13 United States of America	Continuation	Issued	08/477484	06/07/1995	5756699	05/26/1998
14 United States of America	Continuation	Issued	08/839765	04/15/1997	6146631	11/14/2000
15 United States of America	Continuation	Issued	09/711485	11/13/2000	6649742	11/18/2003
16 United States of America	Continuation	Issued	10/717243	11/18/2003	7153932	12/26/2006
17 Patent Cooperation Treaty	Ordinary PCT Application	Expired	PCT/US1994/005348	05/12/1994		

CLFR:264

TITLE: POLYNUCLEOTIDES ENCODING GELONIN SEQUENCES

Client Reference No.: NULL

Gary M. STUDNICKA
Marc D. BETTER
Stephen F. CARROLL

Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1 United States of America	Standard via PCT	Issued	08/646360	05/12/1994	5837491	11/17/1998
2 United States of America	Continuation	Issued	09/136389	08/18/1998	6146850	11/14/2000
3 United States of America	Continuation	Issued	09/610838	07/06/2000	6376217	04/23/2002
4 United States of America	Continuation	Lapsed by inaction	10/127,890	04/23/2002		
5 United States of America	Continuation	Lapsed by inaction	10/613,755	07/12/2003		

CLFR:321

TITLE: COMPOSITIONS AND METHODS OF USE OF AGENTS TARGETING IGFBP2

Summary: Fusion constructs comprising an inhibitor of IGFBP2 (e.g., IIP45) fused to gelonin are provided. These fusion constructs may be used to treat various cancers, such as melanomas. The data shows that IGFBP2 ligands can internalize into tumor cells and carry a toxin for targeting cancers which overexpress IGFBP2. IGFBP2 targeting ligands which may be used with the present invention include exons 5-9 of IIP45 and/or an antibody targeting IGFBP2. IIP45 also has anti-cancer properties.

Client Reference No.: NULL

Michael G. ROSENBLUM
Lawrence CHEUNG
FULLER, GREG
Wei ZHANG

Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
1 United States of America	Provisional	Application Filed	61/108385	10/24/2008		

Key Publications

Gelonin Sequence

1: [Rosenblum MG, Kohr WA, Beattie KL, Beattie WG, Marks W, Toman PD, Cheung L.](#)

Amino acid sequence analysis, gene construction, cloning, and expression of gelonin, a toxin derived from *Gelonium multiflorum*.

J Interferon Cytokine Res. 1995 Jun;15(6):547-55.

HuM195/rGel for treatment of AML and Myelodysplastic Syndrome (MDS):

1: [Duzkale H, Pagliaro LC, Rosenblum MG, Varan A, Liu B, Reuben J, Wierda WG, Korbling M, McMannis JD, Glassman AB, Scheinberg DA, Freireich EJ.](#)

Bone marrow purging studies in acute myelogenous leukemia using the recombinant anti-CD33 immunotoxin HuM195/rGel.

Biol Blood Marrow Transplant. 2003 Jun;9(6):364-72.

2: [Pagliaro LC, Liu B, Munker R, Andreeff M, Freireich EJ, Scheinberg DA, Rosenblum MG.](#)

Humanized M195 monoclonal antibody conjugated to recombinant gelonin: an anti-CD33 immunotoxin with antileukemic activity.

Clin Cancer Res. 1998 Aug;4(8):1971-6.

3: [Xu Y, Xu O, Rosenblum MG, Scheinberg DA.](#)

Antileukemic activity of recombinant humanized M195-gelonin immunotoxin in nude mice.

Leukemia. 1996 Feb;10(2):321-6.

4: [McGraw KJ, Rosenblum MG, Cheung L, Scheinberg DA.](#)

Characterization of murine and humanized anti-CD33, gelonin immunotoxins reactive against myeloid leukemias.

Cancer Immunol Immunother. 1994 Dec;39(6):367-74.

Melanoma Treatment Products: ScFvMel/TNF-alpha, ScFvMel/rGel and ScFvMel/Granzyme

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Other Constructs

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Product Candidate: scFvMel/rGel

Indications: Breast Cancer and Melanoma

Stage: Pre-clinical

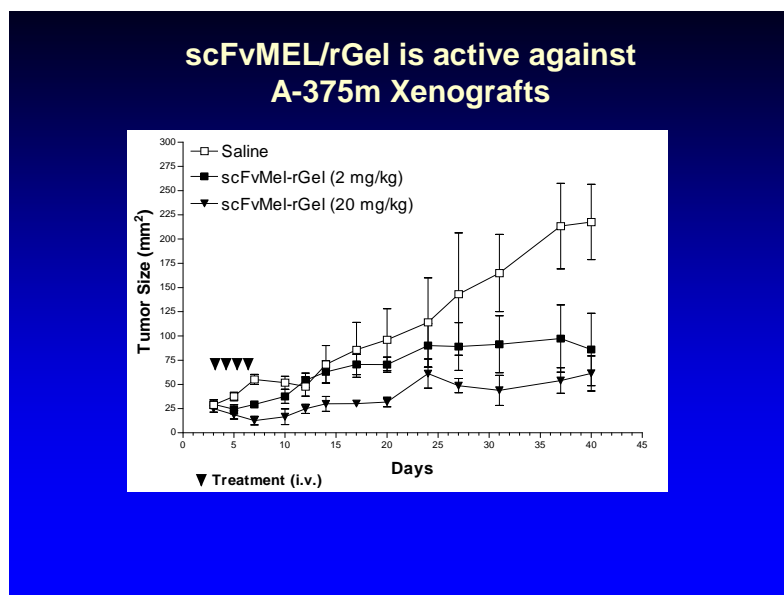
Overview: scFvMel/rGel is currently being preclinically developed for the treatment and prophylaxis of metastatic melanoma and a molecularly-defined subset of breast cancers.

scFvMel/rGel is an unglycosylated, low molecular weight (46 kDa) recombinant fusion protein. scFvMel/rGel consists of a single chain antibody fragment (scFv) genetically fused to the toxin payload rGel (recombinant gelonin). It can be readily manufactured inexpensively and efficiently in *E. coli* fermenters.

The construct uses a small flexible linker between the variable domains of the heavy and light chains of the antibody ZME-018 attached via a tether (G4S) to the recombinant gelonin. This molecule has the binding properties of the original anti-melanoma antibody and contains biologically active toxin.

The original antibody binds to a surface glycoprotein (gp240) found on over 80% of human melanoma cell lines and biopsy specimens. While most melanomas are currently treated surgically, metastatic disease with a highly negative prognosis remains a significant risk for such patients. New agents for both the prophylaxis and treatment of metastatic melanoma are currently needed. Recent studies also have demonstrated that this antigen is expressed on 67% (44/66) of lobular breast carcinomas studied. Lobular breast carcinomas account for about 15% of all breast cancers and appear to be an immunologically distinct subclass from HER2+ breast cancers.

Pre-clinical studies on A-375 Melanoma cell lines and A-375 tumor bearing nude mice strongly indicate that scFvMel/rGel has potent cytotoxic activity both in vitro and in vivo and is an excellent candidate for clinical development. scFvMel/rGel also has a sister molecule, scFvMel/TNF, which uses human TNF- α instead of recombinant gelonin as its payload. Although not as far developed clinically, scFvMel/TNF- α is also showing very good activity in animal models. We have completed most of the pre-clinical development for this product candidate.



Product Candidate: scFvMEL/TNF- α

Indications: Melanoma and other gp240+ tumors

Stage: Advanced Pre-clinical

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

ScFvMEL/TNF- α 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- α). TNF-alpha itself naturally forms into a compact trimer in solution. Analysis of ScFvMEL/TNF- α also demonstrates that the fusion construct exists in solution as a trimer of ~135 kDa. ScFvMEL/TNF- α 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- α has potent cytotoxic activity both in vitro and in vivo and is an excellent candidate for clinical development. ScFvMEL/TNF- α also has a sister molecule, ScFvMEL/rGel, which uses recombinant gelonin instead of human TNF- α as its payload.

We have completed most preclinical development on scFvMEL/TNF- α 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)

