Tumorase™: *In vivo* efficacy data for novel localized proteinase treatment applicable to solid tumors of varied tissue origins

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Developing Cures for Cancer

Abstract

To facilitate the development of non-invasive solid tumor cancer treatments, BioMedicure reports here the development of a specific proteinase treatment that when injected locally into solid tumors elicits a substantial tumor remission. Reported here is data indicating that intratumoral injection of Tumorase™ (T101, BioMedicure, San Diego, CA), a novel proteinase formulation, into solid tumors of various tissue origin destroys tumors by digesting the tumor solid-structure, killing cancer cells, including cancer stem cells, locally while not effecting normal tissues. The best route of Tumorase™ biochemical delivery to tumors is intratumoral injection by a needle. Tumorase™ works best when intratumorally injected at about 50-80% of solid-tumor volume delivered not by bolus but at about 200 uL per minute. The success rate of eliminating sparsely vascularized solid-tumors 50-400 mm^3 in volume is high (over 70%). Multiple treatments can be applied to further diminish tumor mass. Data will be presented for the following tumor models, the human melanoma, prostate, breast, bronchioalveolar carcinomas and colon adenocarcinomas xenografts.

Objectives

- When a cancerous growth is found early and is accessible by a surgical knife, surgery is still the recommended treatment.
- However, if not accessible or there are multiple tumors, then, Tumorase™ would be a viable option, a local therapy that can be utilized for multiple tumors' elimination.
- Tumorase™ contains proteases that cleave the extracellular matrix, including vital proteins required for cell survival, leading to cancer cell death.
- Presented here is *in vivo* data indicating the efficacy of this novel proteinase preparation for the elimination of solid tumors of multiple tumor classes by destroying tumors' solid structure and killing cancer cells by cleaving the extracellular domains of vital cell membrane proteins.
- The wide range of solid cancerous tumors eliminated suggests a potential wide clinical applicability.

Further Reading

Qian, Y. 2008. Proteinases destroy cancer tumor's solid structure and kill cancer cells locally. United States of America, Patent and TradeMark Office publication No: 20080014190

Materials & Methods

A. Tumorase™ Usage

- Route for biochemotherapy administation is intratumoral delivery by a needle with a gauge 20-27.
- For determining appropriate dosage the tumor volume is first estimated by 3-dimensional measurement and calculation.
- Maximum efficacy has been observed when administered at 50-80% of solid-tumor by volume delivered slowly at a rate of about 200 uL per minute for solid-tumors of volume from 50-400 mm^3.
- Second injections useful if full remission not observed with first injection, usually highly vascularized tumors.

B. Tumorase™ Safety Tests

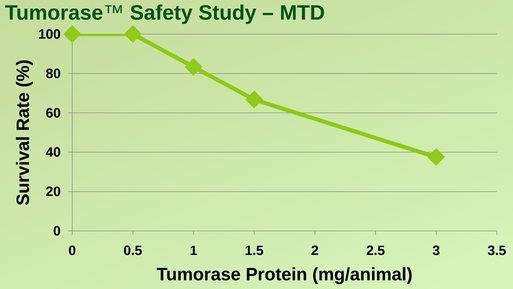
- Tumorase™ in the amount of 0, 0.5, 1, 1.5 and 3 mg were injected sub-Q to 5 groups of nude mice each composed of 3 males and 3 females, except 3 mg/ml with 4 males and 4 females. The MTD determined of 0.5 mg is twice the dose of 0.25 mg used routinely. Nude mice (athymic NCR nu/nu, ratio 1:1 male to female, 3-4 weeks old) were purchased from Simonsen Laboratories Inc. (Gilroy, CA) and directly delivered to a CRO vivarium facility. All procedures comply with IACUC regulations.
- The LD_{50} is about 2 mg per mouse delivered sub-Q. The cause of the death appears to be blood loss. High concentrations of Tumorase™ may kill actively dividing cells and may disrupt cells of arterial and capillary walls and valves of vein as well.

C. Tumorase™ Efficacy Tests

- Cultured cancer cells harvested with trypsin, 4 x 10^6 per animal, were injected (sub-Q) to over 100 nude mice over 80 wild-type mouse for tumor initiation per approved animal experimental procedures.
- Tumorase™ with various concentrations were applied intratumorally at the initial session to multiple primary tumors and if complete remission is not observed with first application the procedure was repeated up to five times.
- Since inhibitory factors in serum inhibit the activity of Tumorase™ and the active molecule is very large, the success rate of eliminating a solid-tumor locally is high (over 70%). However, for the same reason, the success rate of eliminating a highly vascularized soft or liquid tumor is low (below 30%).

Results

A. Tumorase™ Safety Study – MTD



≤0.25 mg utilized for efficacy studies

B. Tumorase™ Efficacy

- **Xenograft CRL-1676 Melanoma**

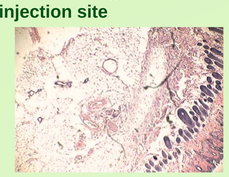
Tumor mass present
~ 100 mm^3

Tumorase™

Two hours post treatment – liquefied tumor fluid exudes from injection site

37 days post treatment

Histopathology at Tumorase™ injection site



No lesions apparent

- **Xenograft CRL-250 Prostate**

C. Tumorase™ eliminated tumors in 135 animals from 7 cancer types

Tumor cell line type	Treated Tumor #	Eliminated Tumor #	Efficacy (%)*
Melanoma	20	20	100
Breast adenocarcinoma	18	18	100
Breast ductal carcinoma	12	12	100
Colon adenocarcinoma	14	14	100
Prostate carcinoma	15	15	100
Bronchioalveolar	41	41	100
Lung carcinoma	15	15	100

*Efficacy refers to tumor elimination for 1 to several days after one injection.

Tumorase™ Mechanism

A. Primary tumor disruption response

Tumorase™ intratumoral injection → Proteinase cleaves extracellular proteins keeping membrane intact → high concentration of amino acids creates hyperosmotic environment in the liquefied tumor → Attracts body liquids , the pressure builds up by low water potential , discharge of the liquefied tumor waste via injection point

B. Secondary immune response

Tumorase™ Killed Tumor Cells → **"Foreign cell" recognition by immune system** → Cancer cell specific antigen identification → **Immune responses to kill residual cancer cells**

Summary

- Tumorase™ is a local biochemotherapy
- Destroys the solid structure of a tumor
- Kills all cancer cells, including cancer stem cells, within a solid tumor
- Effective within an hour
- Solid tumor specific
- Has minimal side effects
- Has potential to combine with other existing therapies

- Completed a pilot-scale manufacturing process
- Developed quality control methods
- Discussed plan for initiating human trials with the FDA in 2008
- Ready to enter clinical trial for companion animal uses within 12 months

Discussion

Tumorase™ over surgery

- Minimal sacrifice of normal tissue surrounding solid tumors
- Removes solid tumors where surgery cannot be performed
- Eliminates multiple solid tumors at the same time

Tumorase™ over radiotherapy, chemotherapy or immunotherapy

- Kills all cancer cells, including cancer stem cells, within solid tumors
- Does not mutate normal cells
- Eliminates solid tumors specifically
- Eliminates all types of solid tumors
- Shortens treatment time by more than three weeks