# ROSE BENGAL INDUCES DUAL MODES OF CELL DEATH IN MELANOMA CELLS AND HAS CLINICAL ACTIVITY AGAINST MELANOMA

MOUSAVI SH,[1] ZHANG XD,[2] WACHTER E,[3] HERSEY P[2]

[1]Mashad University of Medical Science,Mashad, Iran., [2]University of Newcastle,NSW,Australia, [3]Provectus Pharmaceutics,Knoxville,Tennesee,USA,

## Abstract

**Rose Bengal has been used to assess hepatic function,opthalmic disorders and as a photosensitiser in treatment of skin lesions. In this study RB was tested in-vitro in the absence of light for its effects on melanoma cells and mechanism of its effects. In addition a patient was treated by intralesional injection of a sc metastasis with PV-10(RB).**

**Methods**

**A panel of melanoma cells were exposed to RB at different concentrations and for varying periods and effects studied by morphology apoptosis assays and MTT assays**

**Results.**

**RB induced cell death in melanoma cells but not fibroblasts. Death was predominantly due to necrosis but 2 lines also underwent apoptosis that was dependent on activation of caspases. Cell death was not due to release of Reactive oxygen species.RB is taken up into lysosomes and it is probable (but not proven) that cell death results from release of Cathepsins.**

**The patient who had chemotherapy radioresistant recurrent disease in the head and neck area had rapid necrosis of the injected metastasis and this as well as 3 uninjected sc metastases under went complete remission over a period of several months**

**Conclusion**

**Further studies to understand the mechanism of action and its role in clinical management of melanoma are warranted**

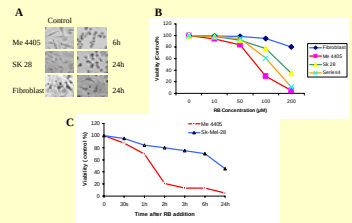



Fig.1. Effect of RB on Morphological changes (A) and Cell Viability (B) in Melanoma cell lines and Fibroblast. Time Course of RB-induced Toxicity (C)

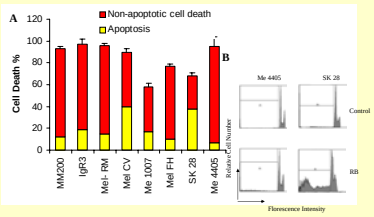



Fig.2. A: RB (200 µM) Induces both Non-Apoptotic and Apoptotic Cell Death in Melanoma Cells after 24h
B: Flow Cytometry Histograms of Apoptosis Assays by the PI Method in . SK 28 and Me 4405 Cells Treated with RB (200 µM) for 24 h

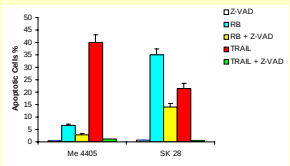



Fig.3. RB (200 µM) Induces Apoptosis of Melanoma through Caspase-Dependent and -Independent Pathways

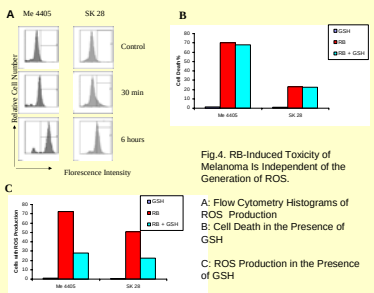



Fig.4. RB-Induced Toxicity of Melanoma Is Independent of the Generation of ROS.

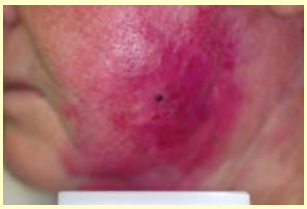
A: Flow Cytometry Histograms of ROS Production
B: Cell Death in the Presence of GSH

C: ROS Production in the Presence of GSH

## Clinical Effects of Intralesional PV-10 ( RB)

The following photos illustrate complete remission of sc metastases that recurred following surgery and irradiation.

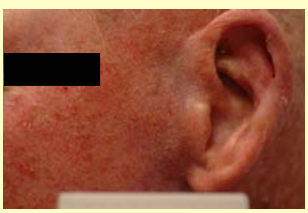
### After Injection of PV-10



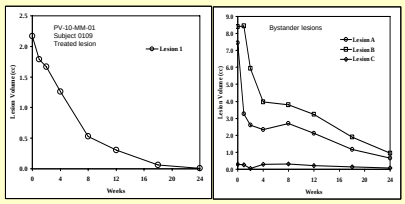
### Two months .Necrosis of injected lesion

### Six Months.Clearance of injected lesion

### Ten months. Clearance of bystander lesions

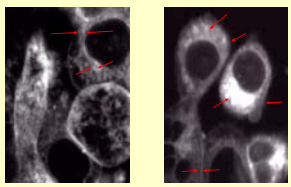


## CHANGE IN VOLUME OF PV-10 INJECTED AND BYSTANDER LESIONS

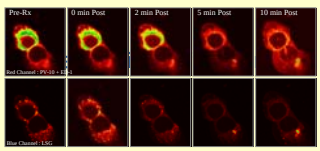



## Mechanism of Action?

- RB is taken up by tumor cells and not normal cells.
- RB localizes in Plasma membranes, nuclear membranes,lysosomes and ? Golgi



- *Internalization in membranes of tumor cells*
- *Accumulation in perinuclear organelles - lysosomes*
- *Exclusion from nucleus*




- *PV-10 activation precipitates lysosomal release*
- *Loss of cell morphology occurs within 5-10 min*

WHY ARE BYSTANDER METASTASES RESPONDING?

Perhaps due to Immune responses?

### Potential Systemic Benefit

**PV-10 Autolysis can stimulate Anti-Tumor Immunity**

- ***Tumors often mask antigenic material***
  - ***Prevents recognition of aberrant cells***
  - ***Allows tumors to grow unchecked***

***Autolysis of tumor cells leads to impulse exposure of immune system to antigenic tumor material***

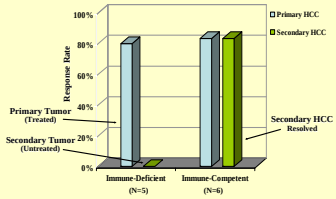
***PV-10 doesn't denature tumor antigens***

***Immune system not compromised by localized Rx***

***Tumor-specific immune response can result***

## Resolution of Contralateral Tumor

(After Treatment of Primary Tumor)




## Possible Mechanism

- **Untreated tumors exhibit high levels of granulocytes (basophils, eosinophils and mast cells) in tissue surrounding tumors**
- **PV-10 treatment results in increased levels of mononuclear tumor-infiltrating lymphocytes**
- **Release of tumor antigens to local antigen-presenting cells may facilitate presentation of appropriate antigenic targets to T and B-cells**
- **Collateral destruction of granulocytes surrounding the tumor may precipitate chemokine release and local inflammation, and could serve an adjuvant role in promoting specific anti-tumor response**

## SUMMARY

**Rose Bengal (PV-10)is taken up by lysosomes in Melanoma cells but not normal fibroblasts.**

**It triggers predominantly necrotic cell death perhaps by release of cathepsins. ROS appear not to be involved.**

**Intralesional injection of a melanoma metastasis resulted in necrotic death of the injected lesion and 3 bystander lesions. The Bystander response may be to induction of immune response?**

**Further investigation of the role of RB in treatment of melanoma is warranted.**