

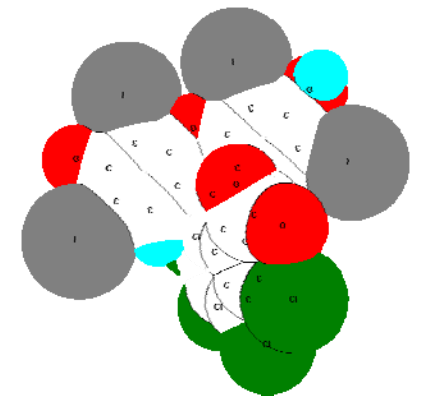
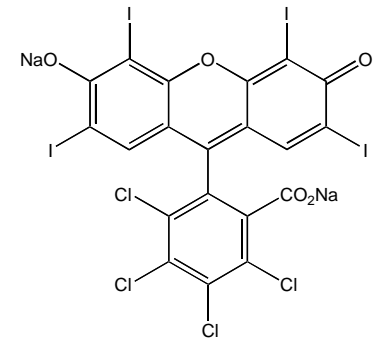
Chemoablation with PV-10

| | |
|-------------------------|---|
| John Thompson | Sydney Melanoma Unit, Sydney, NSW |
| Mark Smithers | Princess Alexandra Hospital, Brisbane, QLD |
| Brendon Coventry | Royal Adelaide Hospital , Adelaide, SA |
| Peter Hersey | Newcastle Melanoma Unit, Newcastle, NSW |
| Merrick Ross | MD Anderson Cancer Center, Houston, TX |
| Sanjiv Agarwala | St Luke's Hospital, Bethlehem, PA |
| David Minor | California Pacific Medical Center, San Francisco, CA |
| Charles Scoggins | University of Louisville, Louisville, KY |
| Eric Wachter | Provectus Pharmaceuticals, Knoxville, TN |

6th International Symposium on Melanoma, NYC 2009

Intralesional Chemoablation with PV-10

- ❑ *Rose Bengal is a Fluorescein derivative synthesised by a German chemist in the late 19th Century*
- ❑ *PV-10 is a sterile, non-pyrogenic solution of Rose Bengal disodium (10% RB)*
- ❑ *PV-10 Targets Neoplastic Tissue*
 - Prolonged retention in tumours
 - Minimal toxicity in normal tissue
 - Selective chemoablation of injected tumour tissue
- ❑ *PV-10 May Elicit a Response in Non-Injected Tumours*
 - Immune-mediated “bystander effect”

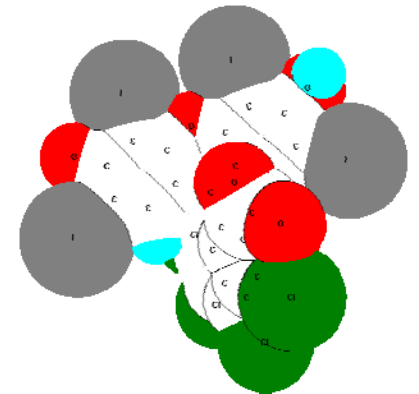
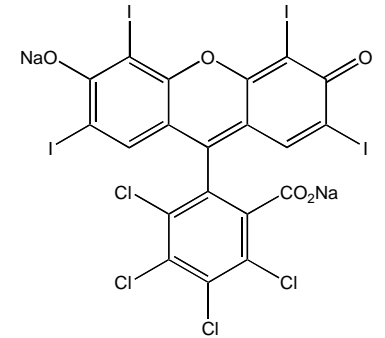


Rose Bengal Disodium (RB)

Intralesional Chemoablation with PV-10

□ *Prior Human Use of Rose Bengal*

- **Established safety history**
 - Not metabolised, has short (30 min) half-life
 - Excretion via liver and kidneys
- **IV hepatic diagnostic**
 - ^{131}I radiolabeled RB: Robengatope[®]
- **Topical ophthalmic diagnostic**
 - Rosettes[®] and Minims[®]



Rose Bengal Disodium (RB)

Broad Spectrum Response to PV-10 in Pre-Clinical Testing

□ *Murine Xenograft Models*

- HCC (Hepa 1-6)
- RAG (CCL-142)
- Melanoma (MeWo, A375, and B16F10)
- Human Breast (MCF-7 and HTB-133/T-47D)
- Human Gall Bladder
- Human Lung (H69Ar – small cell, multidrug resistant)
- Human Prostate (PC-3)

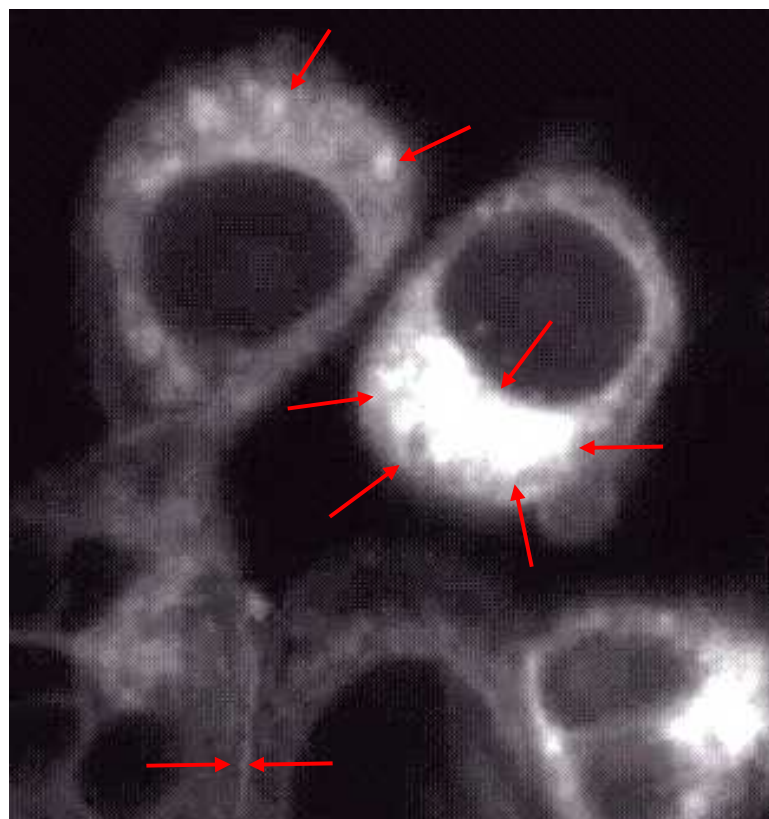
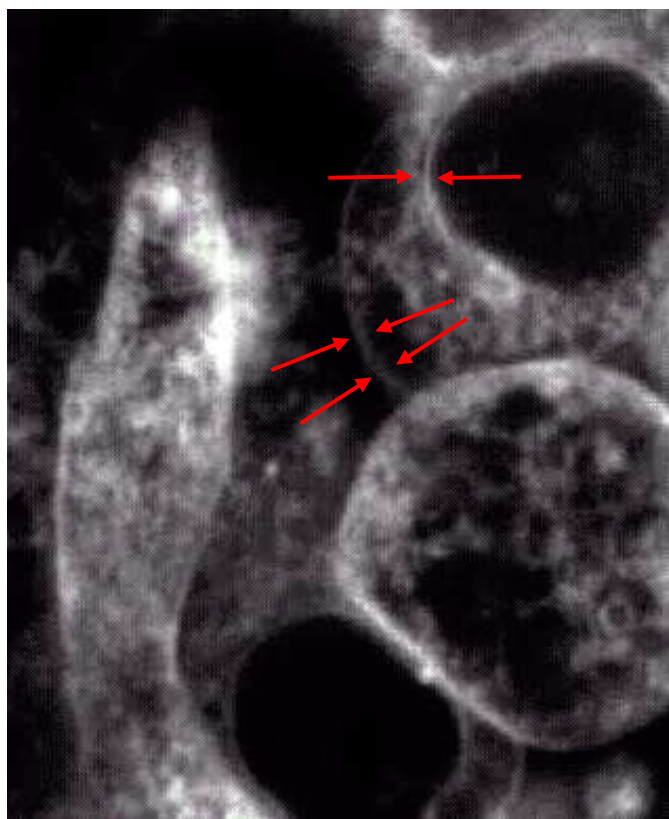
□ *Spontaneous Tumours in Animals*

- Canine recurrent fibrous histiocytoma
- Canine mast cell tumour
- Canine bladder cancer
- Equine melanoma
- Equine sarcoidosis
- Feline squamous epithelial cancer
- Murine breast cancer



PV-10 in PC-3 Prostate Model

Mechanism of Action



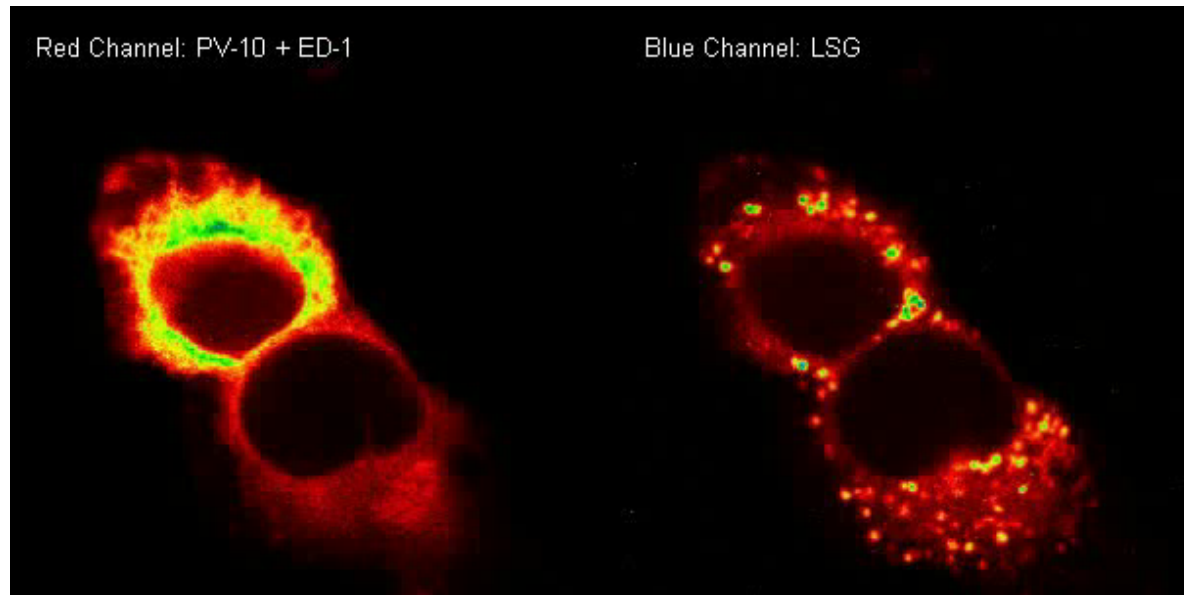
PV-10 in Hepa1-6 HCC

- *Rose Bengal readily transits plasmalemma of cancer cells*
 - Accumulates in lysosomes of cancer cells
 - Excluded from normal cells

Wachter et al., *SPIE Proceedings* 2002; 4622: 112–118

Mousavi, Zhang, Gillespie, Wachter and Hersey, *Mel. Res.* 2006; 16 (supl. 1): S8

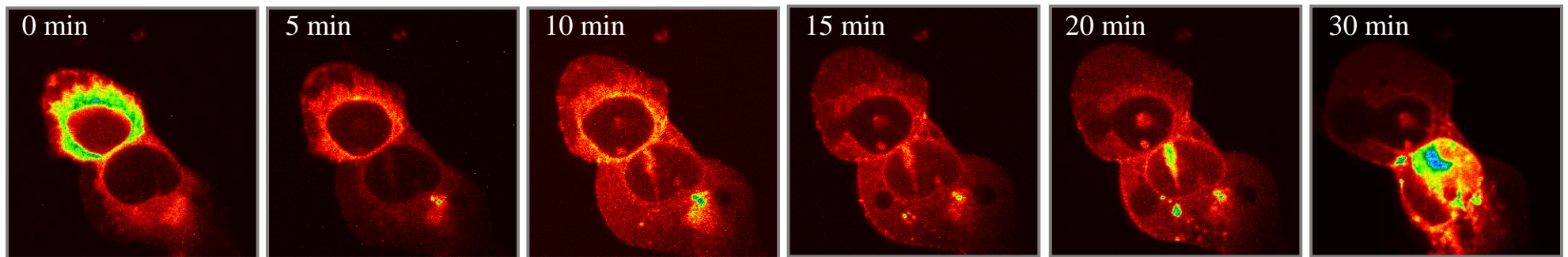
Mechanism of Action



PV-10 in Hepa1-6 HCC
Frames 30 sec apart, total duration ca 60 min

- *PV-10 accumulates in lysosomes of cancer cells*
- *Triggers lysosomal release leading to cell destruction*

Mechanism of Action



PV-10 in Hepa1-6 HCC

□ *Rose Bengal elicits Acute Autophagy of Cancer Cells*

- Triggers immediate lysosomal release
- Void formation and loss of cell morphology within 5-10 min
- Loss of nuclear integrity within 10-30 min
- Complete autophagy within 30-60 min
- Observed in Hepa1-6 HCC, HTB-133 human breast and H96Ar human prostate carcinomas

Bystander Effect

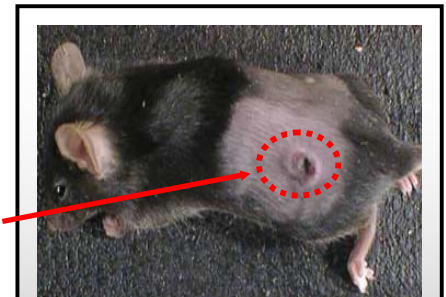
□ *IL PV-10 elicits acute necrosis of treated tumour*

- Ablation quickly destroys bulk of tumor
- RB does not destroy tumor antigens
- Acute exposure to antigenic tumour fragments
- Localized treatment does not compromise immune system

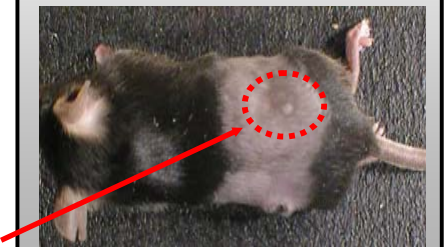
□ *Acute necrosis can trigger immunological response*

- Secondary tumours rejected in immunocompetent animals
- No immune response in immune-compromised animals
- Response is tumour-specific
 - Secondary HCC rejected when primary HCC ablated
 - Melanomas not rejected when primary HCC ablated
- Adoptive transfer of spleen cells can convey immunity

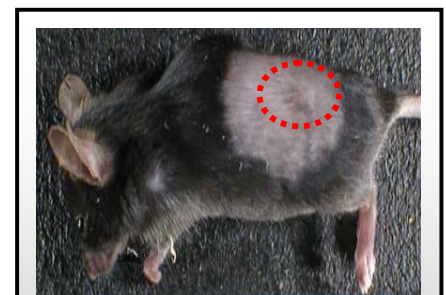
Treated
HCC



Untreated
HCC



• Day 4 Post-Treatment



• Day 10 Post-Treatment

C57BL/6 Immunocompetent Mouse

Bystander Effect

□ *Apparent factors leading to immune mediation*

- Tissue surrounding tumours rich in granulocytes (basophils, eosinophils, mast cells)
- Ablation with PV-10 increases levels of mononuclear tumour-infiltrating lymphocytes
- Release of tumour antigens to local antigen-presenting cells may facilitate presentation of appropriate antigenic targets to T and B-cells
- Collateral destruction of granulocytes surrounding the tumour may precipitate chemokine release and local inflammation, and could serve an adjuvant role in promoting specific anti-tumour response

Clinical Assessment of IL PV-10

□ *Phase 1 Clinical Trial: PV-10-MM-01*

- **20 Subjects with Stage III-IV Metastatic Melanoma**
- **Single IL treatment of 1-20 Target Lesions**
 - PV-10 dosing at 50% of calculated lesion volume
 - Maximum dose of 15 mL PV-10 (1500 mg RB)
 - Observe 1-3 untreated Bystander Lesions
- **12-24 weeks observation**
- **Safety and Objective Response Rate (ORR)**
 - Response assessment based on RECIST

Summary of Phase 1 Trial Results

□ *All 20 subjects treated*

- Median age: 77 (range 40 – 86)
- Total lesions injected: 114 (median 3 per subject)
- Mean PV-10 dose: 2.7 cc (range 0.1 – 15.0 cc per subject)
- 16 subjects (80%) completed study
- 4 subjects (20%) withdrew early due to underlying disease progression

□ *No local SAEs*

□ *1 systemic SAE*

- Grade 3 photosensitivity reaction
 - 15 cc PV-10
 - Concomitant hydrochlorothiazide 12.5 mg QD
 - Prolonged exposure to sunlight after treatment

□ *Local pain in lesions for up to several hours*

- Reported by 75% of subjects

□ *Subject 0001 (female, aged 75)*

- Multiple surgical procedures + ILI in 2 yrs prior to treatment with PV-10
- 3.5 mL PV-10 injected into 3 lesions
- 3 bystander lesions untreated
- Overall CR in injected lesions, SD in bystanders



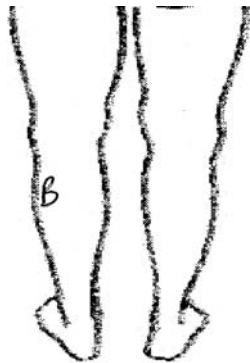
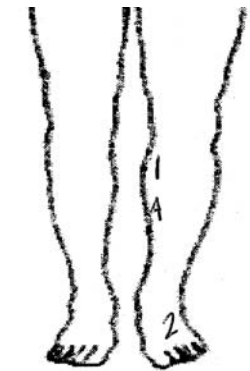
❑ **Subject 0005 (female, aged 83)**

- Multiple surgical procedures in 3 yrs prior to treatment with PV-10
- 4.5 mL PV-10 injected into 3 lesions
- 3 bystander lesions untreated
- Overall PR in injected lesions, SD in bystanders



□ **Subject 0009 (female, aged 84)**

- Numerous surgical procedures in 18 yrs prior to treatment with PV-10
- 2.9 mL PV-10 injected into 2 lesions
- CR in the two injected target lesions



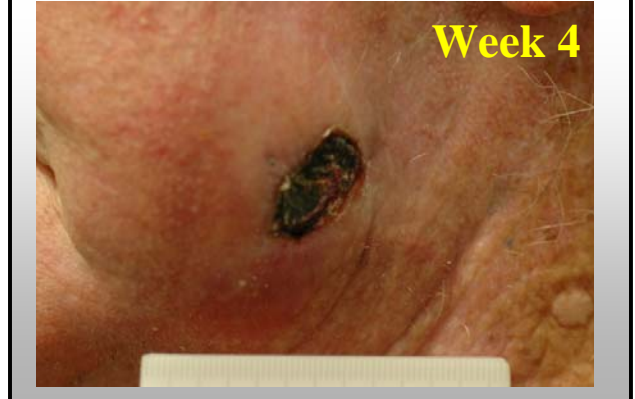
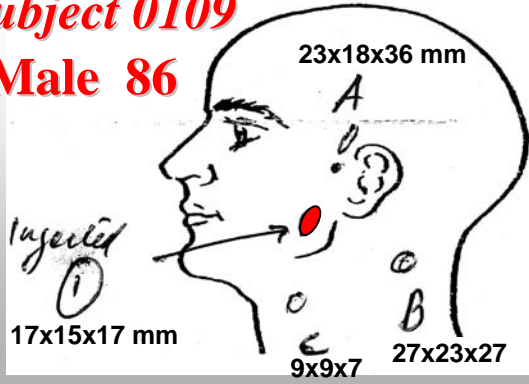
□ *Subject 0009 (continued)*

- Larger bystander lesion stable throughout study interval
- CR of smaller bystander lesion



Subject 0109

Male 86



Phase 1 – Efficacy

| Overall Subject Response (RECIST, N= 20 Subjects graded at end of study interval) | Target Lesions | | Non-Target Lesions | |
|--|-----------------------|------------|---------------------------|------------|
| | N | | N | |
| CR | 4 | 20% | 1 | 5% |
| PR | 4 | 20% | 2 | 10% |
| SD | 7 | 35% | 8 | 40% |
| PD | 5 | 25% | 9 | 45% |
| | | | | |
| CR + PR (ORR) | 8 | 40% | 3 | 15% |
| CR + PR + SD (<i>Locoregional Disease Control</i>) | 15 | 75% | 11 | 55% |

Bystander Response

| Overall Subject Response (Non-Target Lesions) | Subjects with POSITIVE Objective Response of Target Lesions | | Subjects with NEGATIVE Objective Response of Target Lesions | | Fisher Exact |
|--|---|-------------|---|------------|------------------|
| N (subjects) | 8 | | 12 | | |
| CR | 1 | 13% | 0 | 0% | |
| PR | 1 | 13% | 1 | 8% | |
| SD | 6 | 75% | 2 | 17% | |
| PD | 0 | 0% | 9 | 75% | |
| | | | | | |
| CR + PR | 2 | 25% | 1 | 8% | P = 0.537 |
| SD + PD | 6 | 75% | 11 | 92% | |
| | | | | | |
| CR+PR+SD | 8 | 100% | 3 | 25% | P = 0.001 |
| PD | 0 | 0% | 9 | 75% | |

Bystander Response (cont'd)

- Amongst those subjects exhibiting a positive response of their target lesions to PV-10 treatment, 25% experienced an objective response in bystander lesions, and 100% experienced disease control in these lesions
- In contrast, amongst those subjects exhibiting a negative response of their target lesions, only 8% experienced an objective response in bystander lesions, while 75% experienced disease progression in their bystander lesions
- The differences in the rate of locoregional disease control between these two groups was highly significant ($P = 0.001$)

| <i>Adverse Events</i> At Least Possibly Related To Treatment (N=20) | Adverse Events | | | | | |
|---|----------------|-----|-----|------|-------|-----|
| | Mild | Mod | Sev | Life | Total | % |
| <u>Nervous system disorders</u> | | | | | | |
| Headache | 1 | 0 | 0 | 0 | 1 | 5% |
| Insomnia | 1 | 0 | 0 | 0 | 1 | 5% |
| <u>Skin and subcutaneous tissue disorders</u> | | | | | | |
| Photosensitivity reaction | 0 | 0 | 1 | 0 | 1 | 5% |
| Blister | 0 | 1 | 0 | 0 | 1 | 5% |
| Skin ulcer | 0 | 1 | 0 | 0 | 1 | 5% |
| Pruritus | 0 | 1 | 0 | 0 | 1 | 5% |
| Rash pruritic | 0 | 1 | 0 | 0 | 1 | 5% |
| <u>Musculoskeletal and connective tissue disorders</u> | | | | | | |
| Arthralgia | 0 | 2 | 0 | 0 | 2 | 10% |
| <u>General disorders and administration site conditions</u> | | | | | | |
| Injection site pain | 3 | 11 | 1 | 0 | 15 | 75% |
| Injection site cellulitis | 2 | 2 | 0 | 0 | 4 | 20% |
| Injection site pruritus | 3 | 0 | 0 | 0 | 3 | 15% |
| Injection site vesicles | 0 | 2 | 0 | 0 | 2 | 10% |
| Injection site erythema | 1 | 1 | 0 | 0 | 2 | 10% |
| Injection site dryness | 2 | 0 | 0 | 0 | 2 | 10% |
| Injection site edema | 0 | 1 | 0 | 0 | 1 | 5% |
| Injection site inflammation | 0 | 1 | 0 | 0 | 1 | 5% |
| Injection site infection | 0 | 1 | 0 | 0 | 1 | 5% |
| Injection site warmth | 1 | 0 | 0 | 0 | 1 | 5% |
| Injection site photosensitivity reaction | 1 | 0 | 0 | 0 | 1 | 5% |
| Pain | 1 | 1 | 0 | 0 | 2 | 10% |
| Pyrexia | 0 | 1 | 0 | 0 | 1 | 5% |
| <u>Investigations</u> | | | | | | |
| Thyroid function test abnormal | 0 | 1 | 0 | 0 | 1 | 5% |

Phase 1 SAE – Grade 3 Photosensitivity Reaction



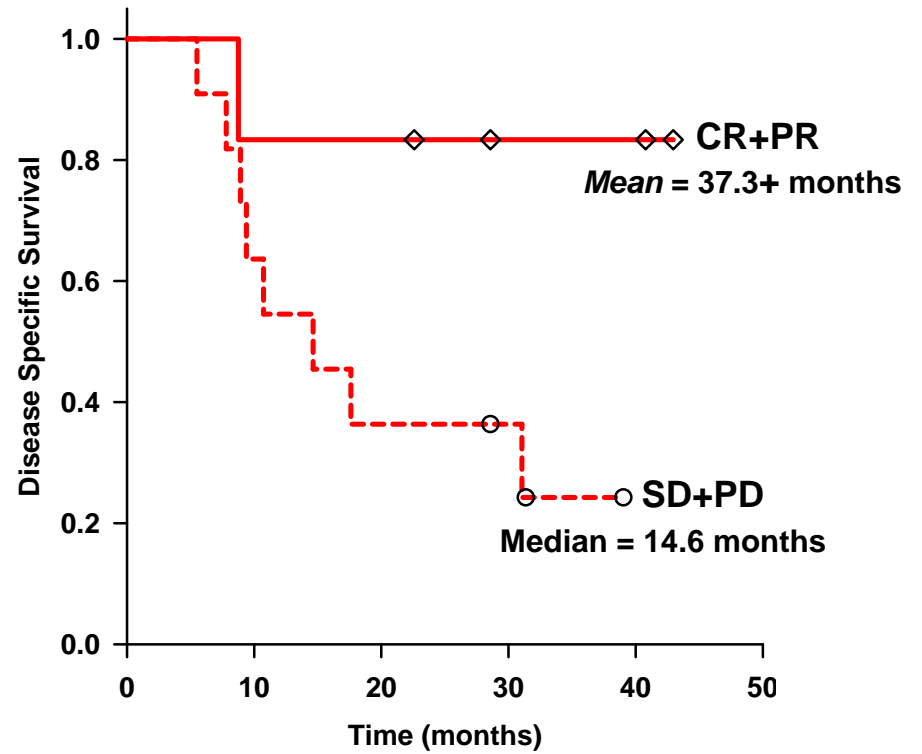
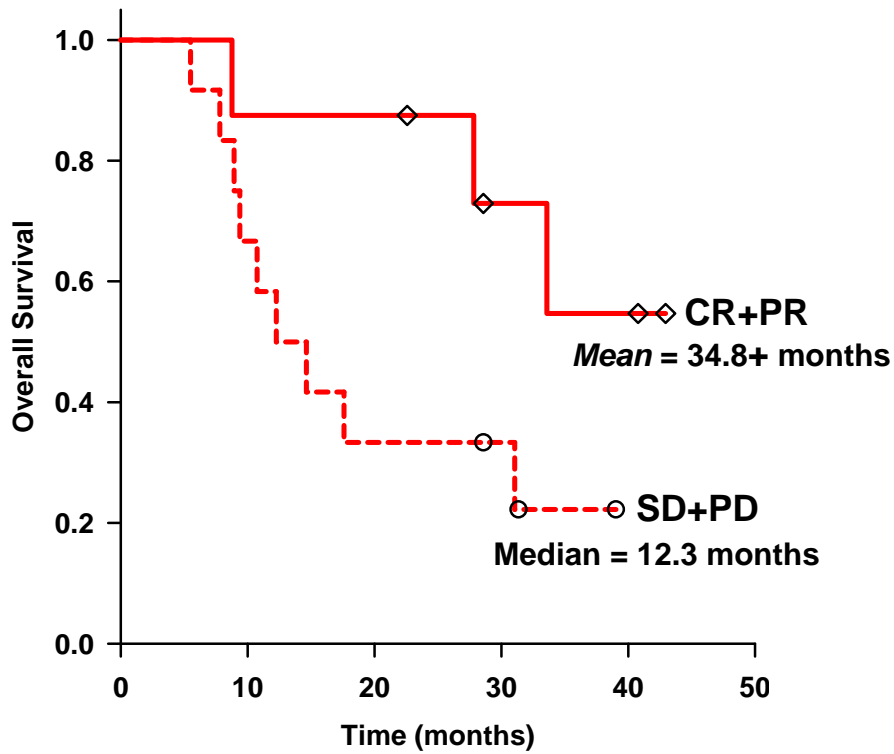
Wiener, Damian and Thompson, *Dermatology* 2008; 216: 361-362



Day 14



Phase 1 – Survival Follow-up



□ Response of Treated Lesions may be a Good Predictor of Long-Term Outcome

Phase 2 Trial – PV-10-MM-02

- **80 subjects with Stage III-IV melanoma**
- **Treatment of 1-10 Target Lesions and up to 10 Non-Target Lesions**
 - Target Lesions must be ≥ 0.2 cm diameter
 - Must biopsy at least one Target Lesion
- **Observe up to 1-2 Bystander Lesions**
 - Typically small or difficult to access
 - Each Bystander Lesion must be biopsied
- **Retreatment (new or partially-responsive lesions) allowed at weeks 8, 12 or 16**
- **Observe for 52 weeks**
 - Outcome (RECIST) assessed on Target Lesions
 - Progression Free Survival
 - Interim Safety Assessment 28 days after 20th and 40th subject treated
 - Interim Efficacy Assessment 24 weeks after 20th and 40th subject treated

Phase 2 – Status

- **First subject enrolled October 2007**
 - 60 subjects enrolled as of 28 February 2009
- **Interim safety assessment on first 40 subjects**
 - Data from first 28 days after initial PV-10 treatment
 - AE profile similar to Phase 1

| Adverse Event | Phase 1 | Phase 2 |
|---|----------------|----------------|
| • Injection Site Pain | 75% | 60% |
| • Injection Site Vesicles | 10% | 30% |
| • Injection Site Oedema | 5% | 28% |
| • Injection Site Swelling | 0% | 18% |
| • Injection Site Erythema | 10% | 10% |
| • Injection Site Cellulitis or Inflammation | 25% | 5% |
| • Injection Site Pruritus | 15% | 2% |
| • Photosensitivity Reaction | 10% | 2% |

Phase 2 – Demographics & Treatment Summary

| | |
|--|-----------------------|
| • Median Age (Range) | 75 yrs (37 – 82) |
| | |
| • Gender | 14 M / 6 F |
| | |
| • Race / Ethnicity: White / Not Hispanic or Latino | 20 (100%) |
| | |
| • AJCC Stage | III (N=16) / IV (N=4) |
| | |
| • Lesions Treated: Total | 275 |
| • Lesions Treated per Subject: Median (Range) | 7 (1 – 20) |
| | |
| • PV-10 Dose per Treatment: Median (Range) | 1.5 mL (0.2 – 15) |
| • PV-10 Treatments: Median (Range) | 1.75 (1 – 3) |

Phase 2 – Preliminary Efficacy

| Best Response (RECIST, N= 20 Subjects graded at Week 8-24) | Target Lesions | | Bystander Lesions | |
|--|-----------------------|------------|--------------------------|------------|
| | N | | N | |
| CR | 6 | 30% | 2 | 13% |
| PR | 6 | 30% | 4 | 25% |
| SD | 4 | 20% | 4 | 25% |
| PD | 4 | 20% | 6 | 38% |
| | | | | |
| CR + PR (ORR) | 12 | 60% | 6 | 38% |
| CR + PR + SD (<i>Locoregional Disease Control</i>) | 16 | 80% | 10 | 62% |

Comparison of Phase 1 vs Phase 2 Efficacy

| Target Lesion Response (RECIST) | Phase 1 | | Phase 2 | |
|---|-----------|------------|-----------|------------|
| | N | | N | |
| CR | 4 | 20% | 6 | 30% |
| PR | 4 | 20% | 6 | 30% |
| SD | 7 | 35% | 4 | 20% |
| PD | 5 | 25% | 4 | 20% |
| | | | | |
| CR + PR (ORR) | 8 | 40% | 12 | 60% |
| CR + PR + SD (<i>Locoregional Disease Control</i>) | 15 | 75% | 16 | 80% |

Phase 2 – Expanded Access

PV-10-MM-02X

- ❑ *TGA Special Access Scheme (Category A) in AUS*
- ❑ *FDA Compassionate Use Guidelines in USA*
- ❑ *Available to Phase 2 Subjects*
 - Evidence of Response to PV-10
 - Disease not completely controlled under Phase 2 Design
 - Allows multiple treatments NLT 28 days apart
 - Anticipate up to 10 Subjects may cross over
 - 3 Subjects currently participating

PV-10-EA-02

- ❑ *Expanded Access for Other Solid, Cutaneous or Subcutaneous Tumors*
 - Numerous inquiries for access to PV-10
 - Trial program at existing Phase 2 centres