

Triamcinolone: Delaying Eye Deterioration

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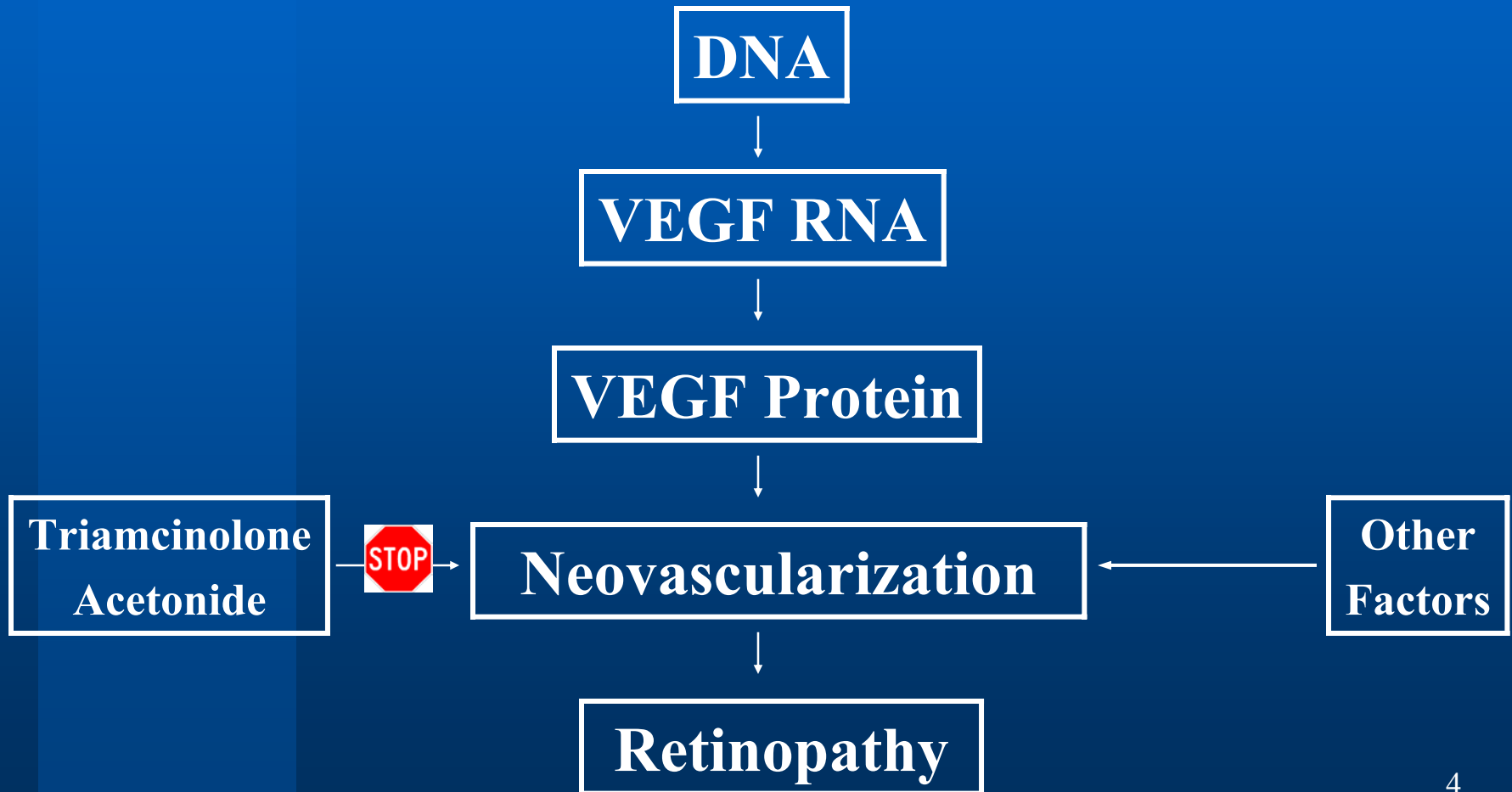
Facts

- **Age-related Macular Degeneration (AMD)** is the leading cause of blindness in the developed Western World
- **Affects over 10 million Americans**
- **Vascular Endothelial Growth Factor (VEGF)** is a protein associated with the development of neovascularization (NV) in:
 - **Age-related macular degeneration (AMD)**
 - **Other retinopathies**

Project Goal

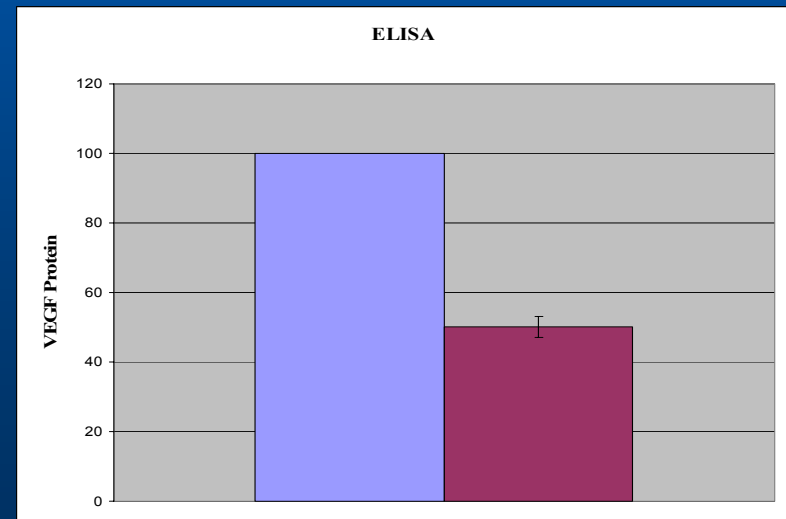
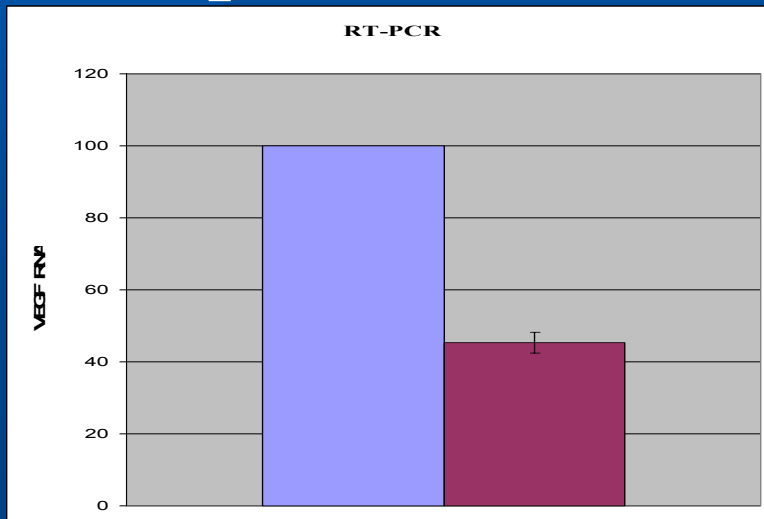
- **To determine if triamcinolone acetonide (TA) decreases cellular concentrations of VEGF**

VEGF & Retinopathy

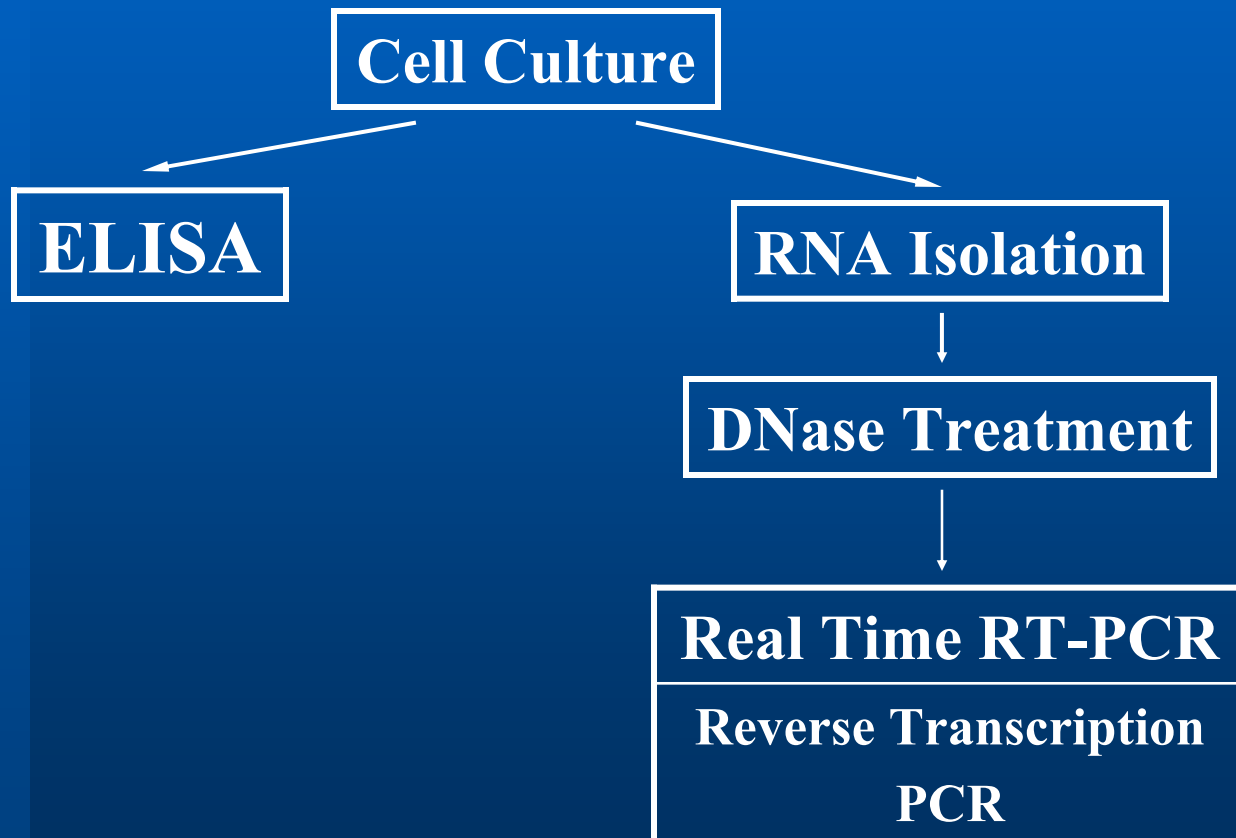


Hypothesis

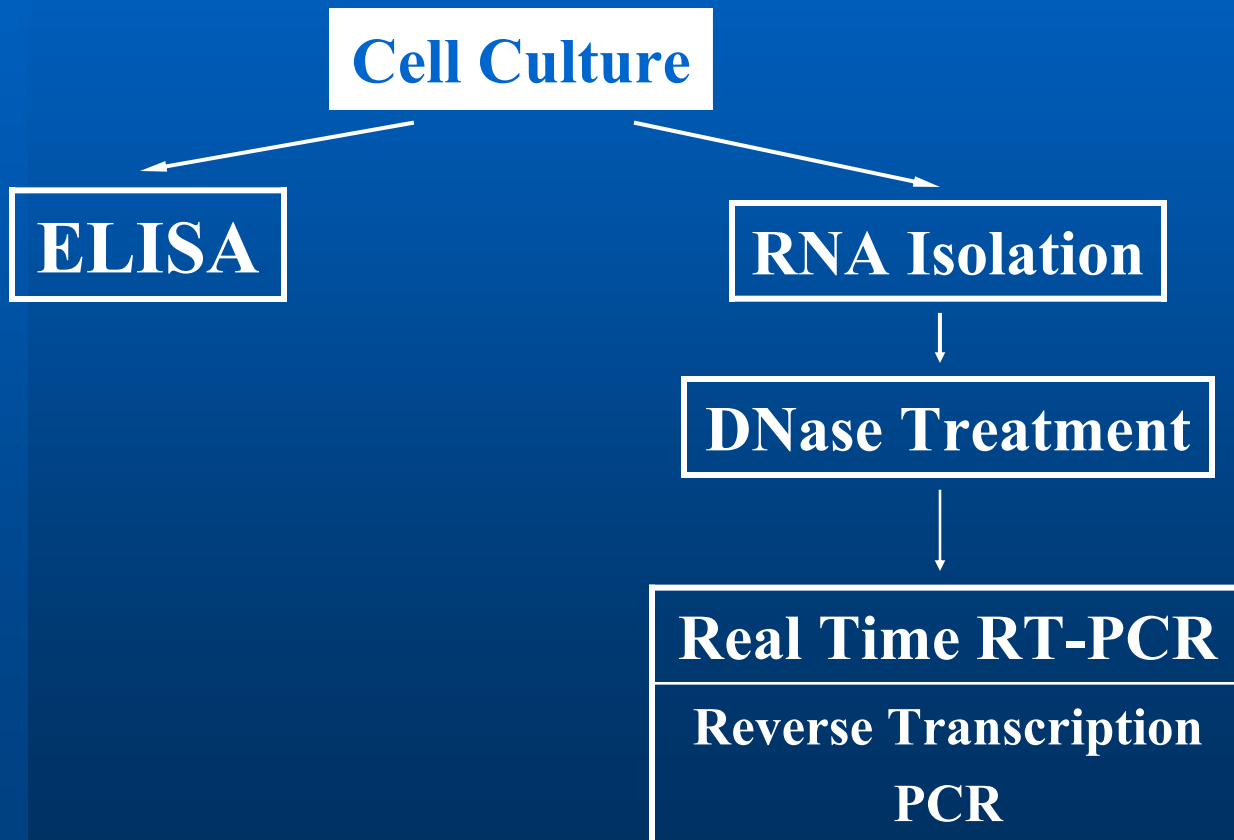
- TA inhibits development of NV by reducing VEGF.
- Expected Data:



Method Overview



Cell Culture



Cell Culture

-	+	+	+	+	+
	CtrlT	1 TA	6 TA	Ctrl5	C5
-	+	+	+	+	+
	CtrlT	1 TA	6 TA	Ctrl5	C5
-	+	+	+	+	+
	CtrlT	1 TA	6 TA	Ctrl5	C5
Just Cell	Just Cell	Just Cell			

-

No Hypoxia

+
CtrlT

Hypoxia
Ctrl for TA

+
1 TA

Hypoxia
1 mg/mL TA

+
6 TA

Hypoxia
6mg/mL TA

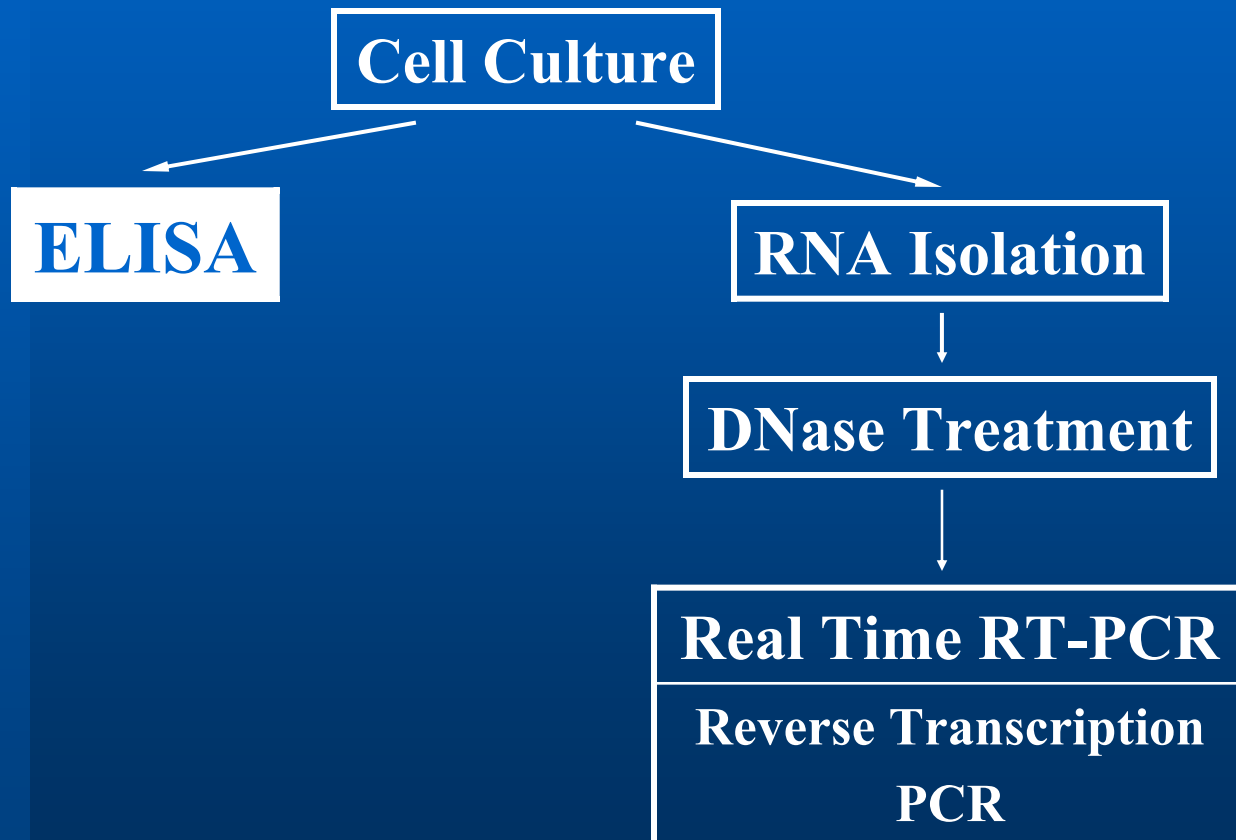
+
Ctrl5

Hypoxia
Ctrl for C5

+
C5

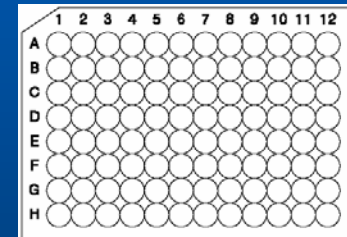
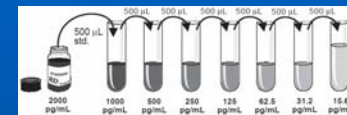
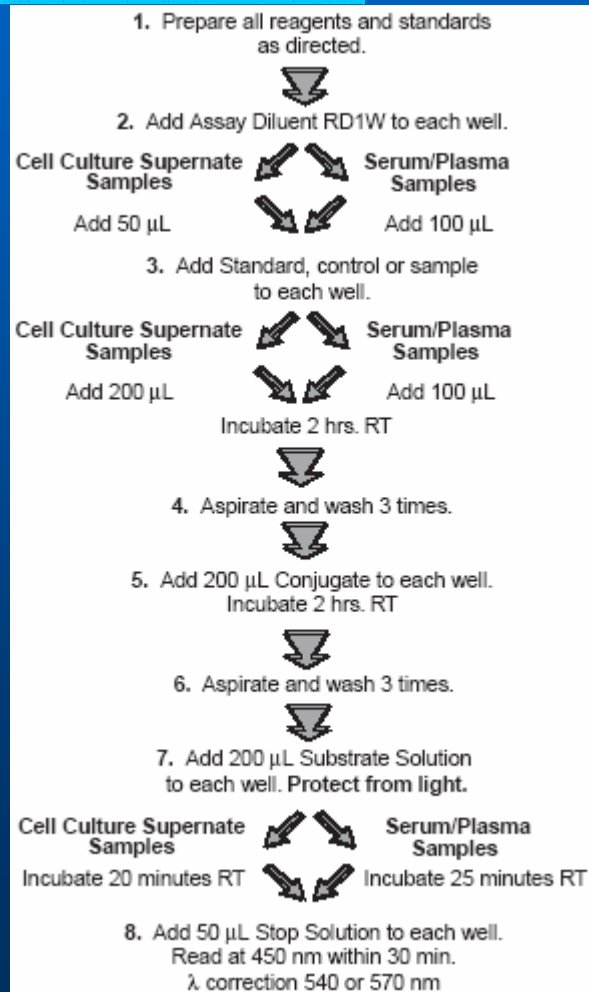
Hypoxia
C5 siRNA₈

Test for VEGF Protein

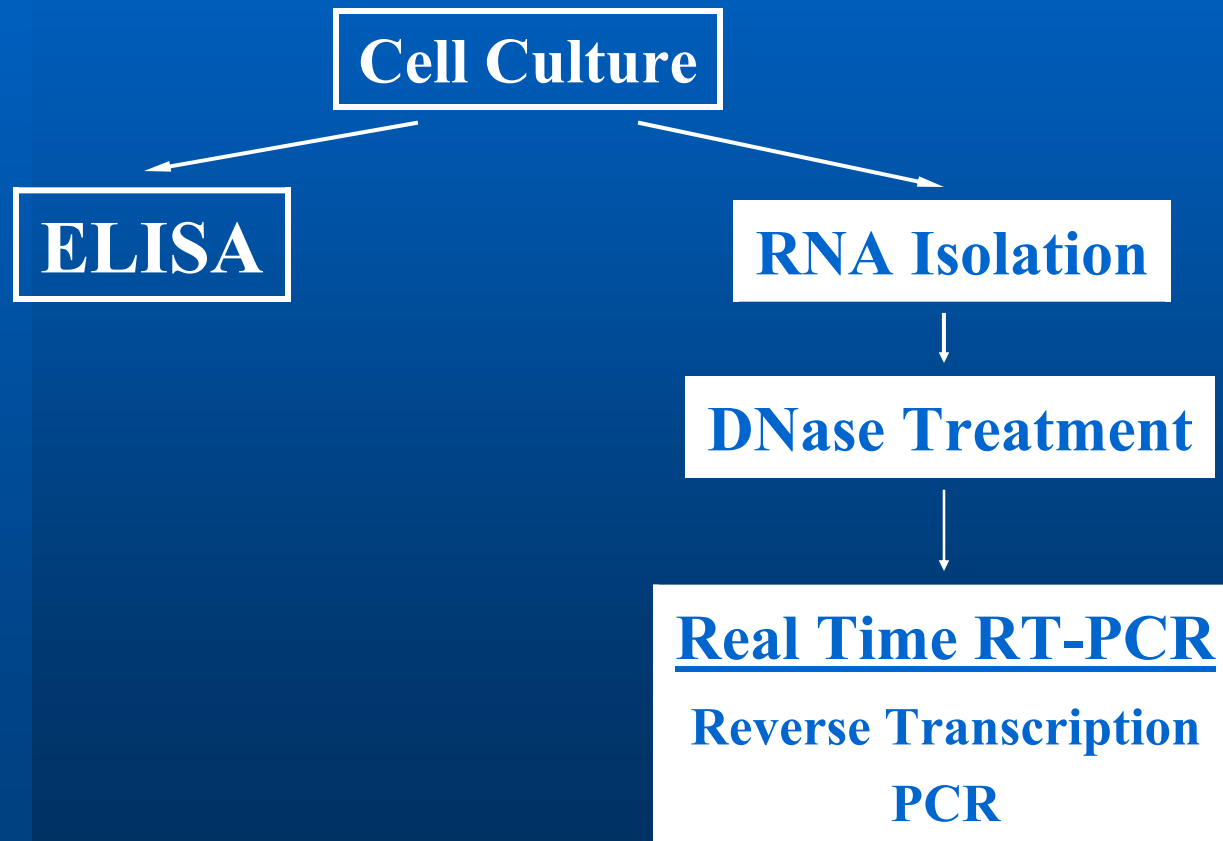


ELISA

● ELISA:



Test for VEGF RNA

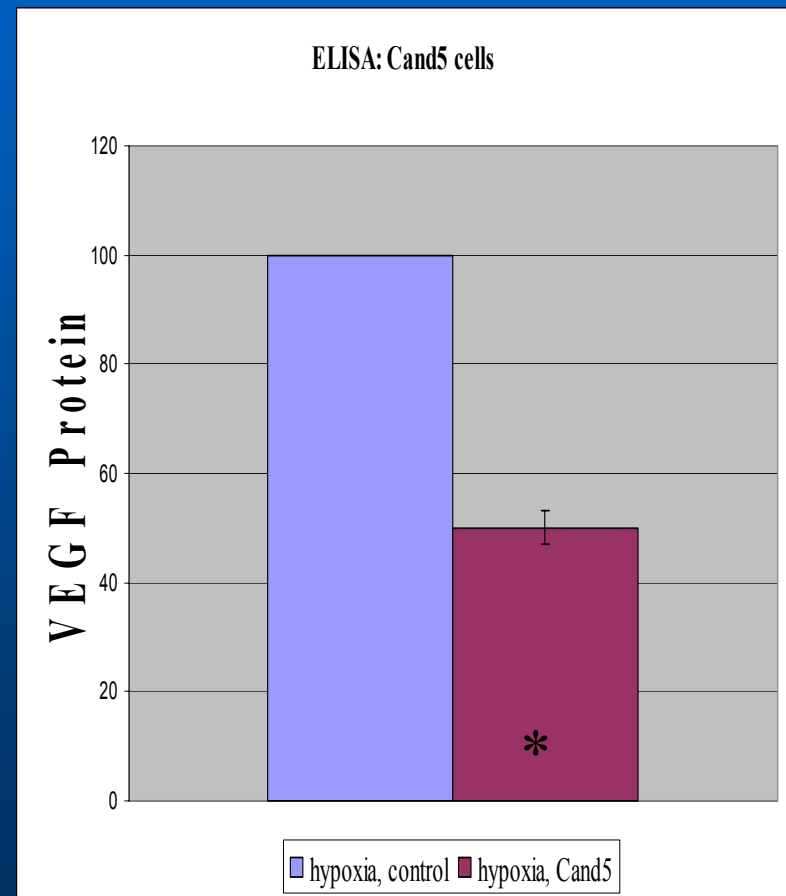
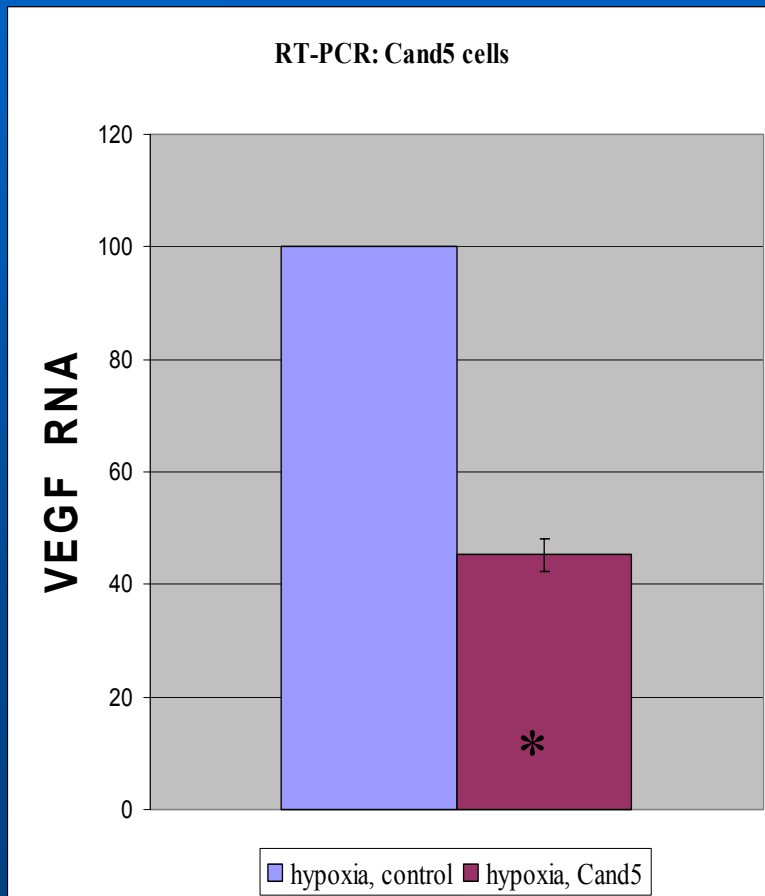


Real Time RT-PCR

VEGF						18s srRNA					
100	25	6.25	- hyp	+ C5	+1 TA	100	25	6.25	- hyp	+ C5	+1 TA
100	25	6.25	- hyp	+ C5	+1 TA	100	25	6.25	- hyp	+ C5	+1 TA
100	25	6.25	- hyp	+ C5	+1 TA	100	25	6.25	- hyp	+ C5	+1 TA
50	12.5		+ctl C5	+ctl TA	+6 TA	50	12.5		+ctl C5	+ctl TA	+6 TA
50	12.5		+ctl C5	+ctl TA	+6 TA	50	12.5		+ctl C5	+ctl TA	+6 TA
50	12.5	NTC	+ctl C5	+ctl TA	+6 TA	50	12.5	NTC	+ctl C5	+ctl TA	+6 TA

Results: Cand5

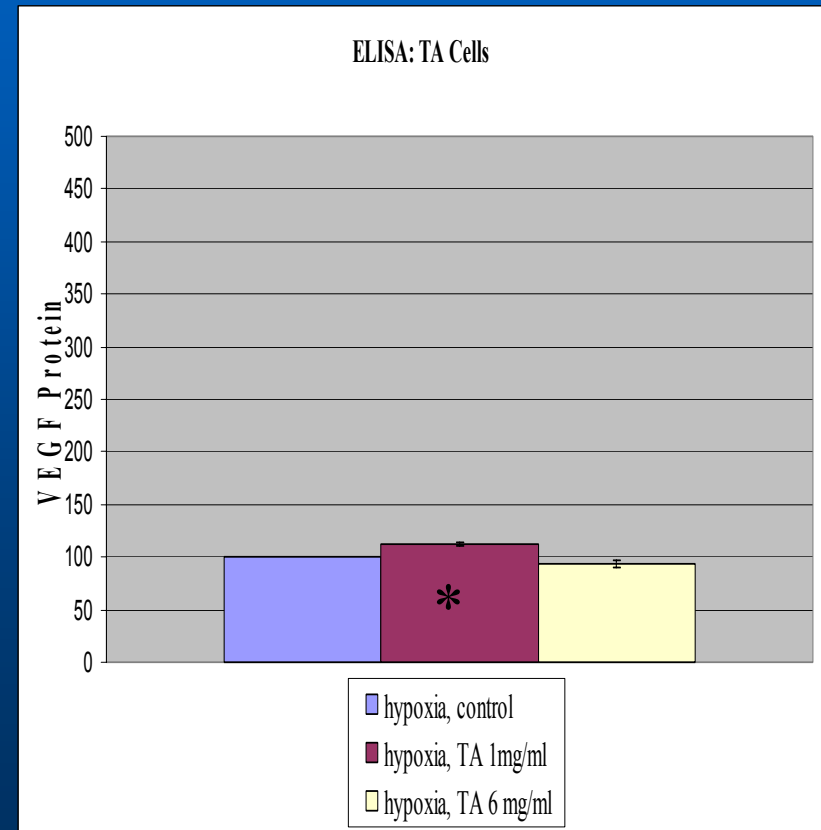
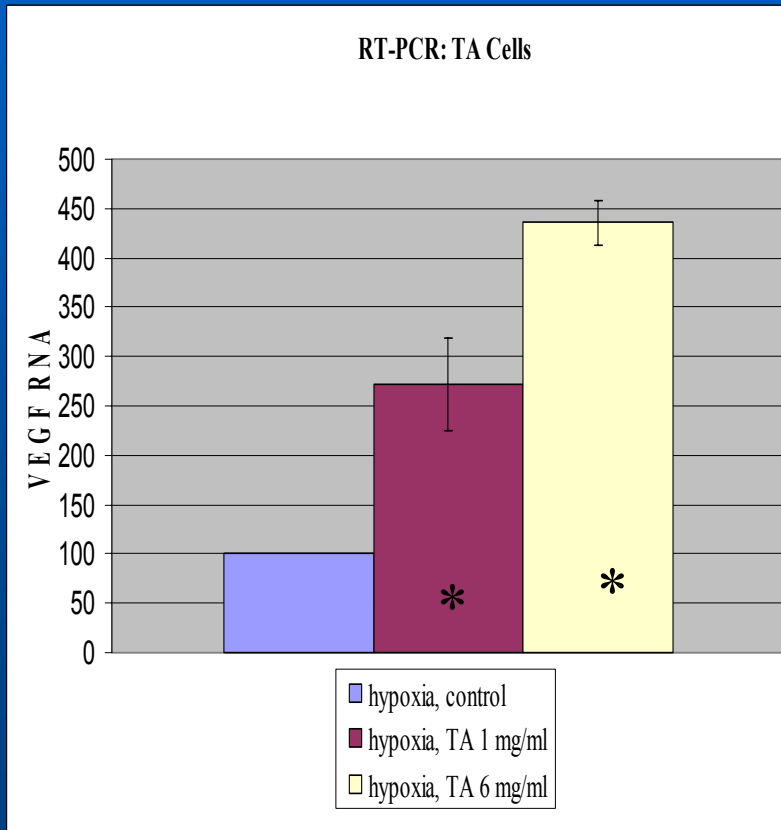
Cand5 decreases VEGF RNA and protein levels as expected



* $P \leq 0.05$ ¹³

Results: TA

TA increases VEGF RNA significantly, but has minimal effect on VEGF protein



* $P \leq 0.05$ ¹⁴

Discussion

- TA does not reduce (may increase) VEGF RNA 24 hours after treatment. The ELISA result does not parallel that of the RT-PCR.
- Possible explanations:
 - Need longer than 24 hours to see effect
 - TA sinks to bottom, increasing TA concentration
 - TA solubility, toxicity, and reaction to heat

FOR MORE INFO...

See [The Toxic and Stress Responses of Cultured Human Retinal Pigment Epithelium \(ARPE19\) and Human Glial Cells \(SVG\) in the Presence of Triamcinolone](#)

AlamarBlue Cytotoxicity Test

- AlamarBlue is a blue dye added to the cells media
- Healthy cells will oxidize it to violet or pink
- Gauge TA toxicity



AlamarBlue Cytotoxicity Test

● Plate Design

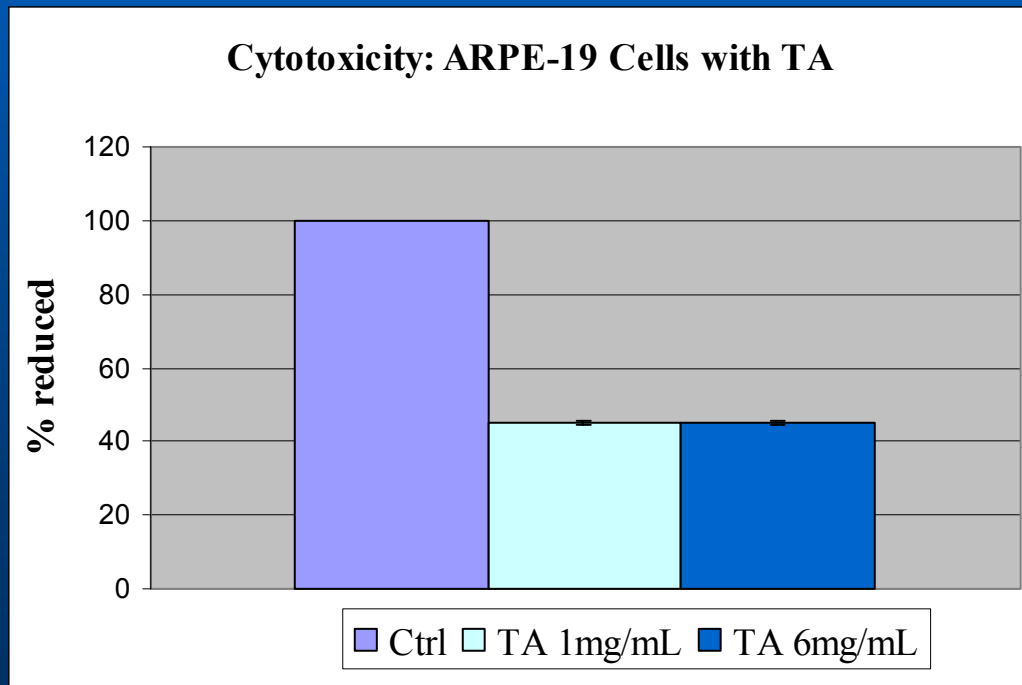
-	+	+	+	+	+
	CtrlT	1 TA	6 TA	Ctrl5	C5
-	+	+	+	+	+
	CtrlT	1 TA	6 TA	Ctrl5	C5
-	+	+	+	+	+
	CtrlT	1 TA	6 TA	Ctrl5	C5
Media Only	Media Only	Media Only	Just Cell	Just Cell	Just Cell

1 TA only	6 TA only	Media +AB			
1 TA only	6 TA only	Media +AB			
1 TA only	6 TA only	Media +AB			
Media Only	Media Only	Media Only			

- 1st trial: TA background reading too high
- 2nd trial: Variance too high
- 3rd trial: Background and variance low

Cytotoxicity Results

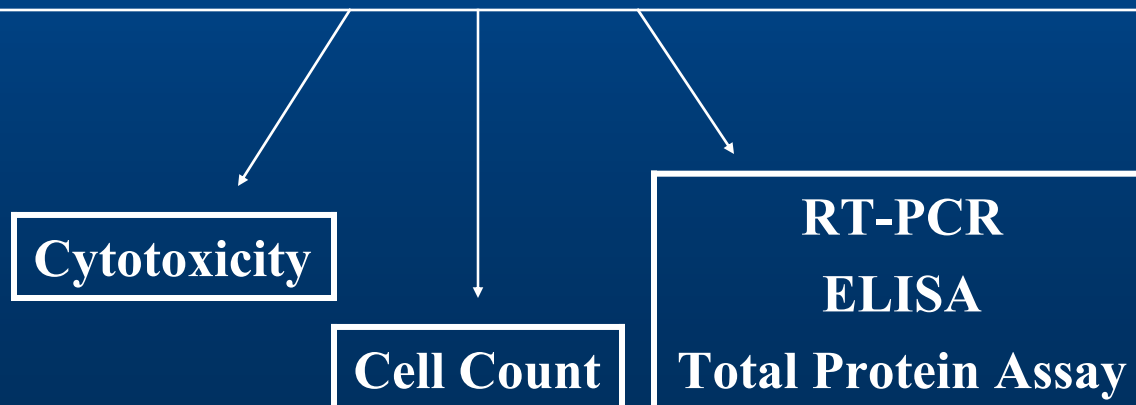
- TA is toxic.



Normalized Cytotoxicity

-	+	+	+	+	+
	CtrlT	.01TA	.1 TA	1 TA	6 TA
-	+	+	+	+	+
	CtrlT	.01TA	.1 TA	1 TA	6 TA
-	+	+	+	+	+
	CtrlT	1 TA	.1 TA	1 TA	6 TA
Media +AB	Media +AB	Media +AB	Just Cell	Just Cell	Just Cell

.01TA only	.01TA only	.01TA only	.01TA only	.01TA only	.01TA only
.1 TA only	.1 TA only	.1 TA only	.1 TA only	.1 TA only	.1 TA only
1 TA only	1 TA only	1 TA only	1 TA only	1 TA only	1 TA only
6 TA only	6 TA only	6 TA only	6 TA only	6 TA only	6 TA only



Normalized Cytotoxicity

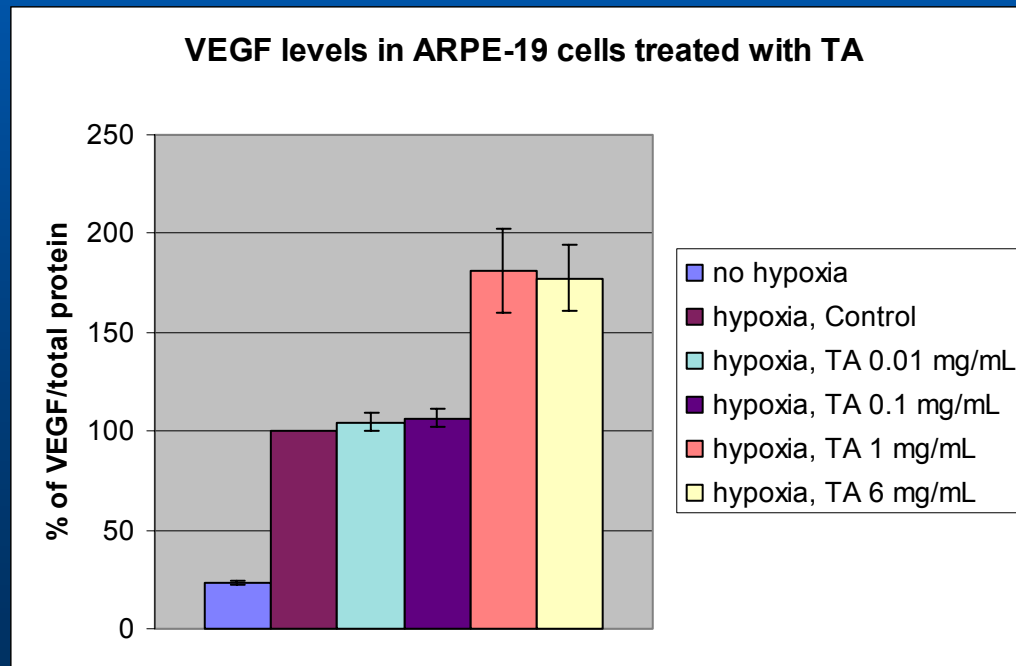
- **Cytotoxicity: Testing lower TA doses**
 - 0.01 mg/mL
 - 0.1 mg/mL
- **Cell Count: Z1 Particle Counter**
 - Normalize VEGF to number of cells per vial
- **RT-PCR**
- **ELISA**
- **Total Protein Assay: BCA Assay**
 - Normalize VEGF to Total protein found instead of srRNA housekeeping gene

Normalized Cytotoxicity

- **Cytotoxicity: Testing lower TA doses**
 - 0.01 mg/mL
 - 0.1 mg/mL
- **BCA Total Protein Assay**
 - Normalize VEGF to Total protein found instead of srRNA housekeeping gene
- **Z1 Particle Counter**
 - Normalize VEGF to number of cells per vial

Normalized to Total Protein

- **ELISA & Total Protein Assay:
TA increases VEGF Protein**



Further Investigations

- **TA vs. VEGF normalized to RNA**
 - RT-PCR
- **TA vs. VEGF normalized to Cell Count**
 - Z1 Particle Counter

Closing Thoughts

- **Age Related Macular Degeneration will cause blindness in 10 million Americans**
- **And we here at the University of Pennsylvania are working towards its solution.**

The End