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Selective Ablation of Senescent and Malignant Cells Using Apoptotic Gene Therapy

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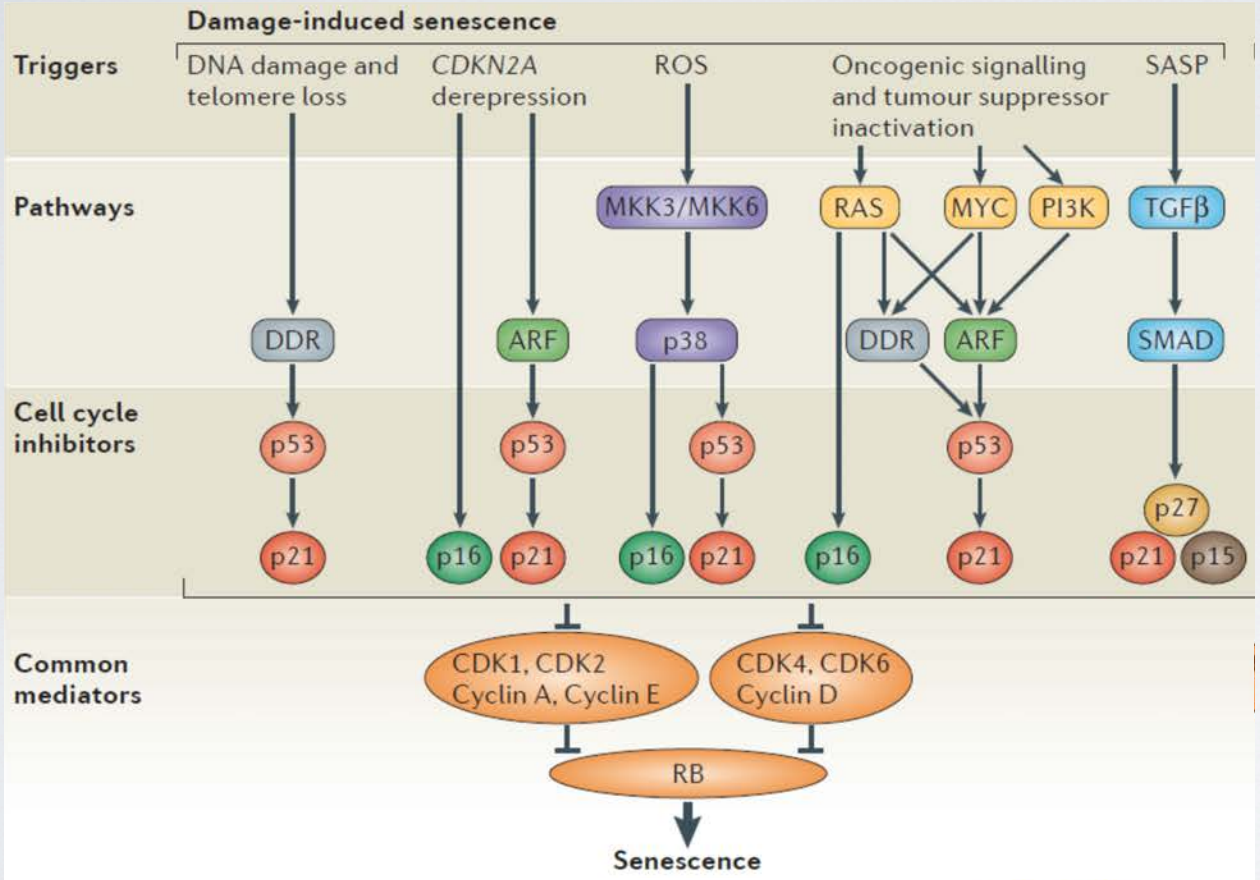
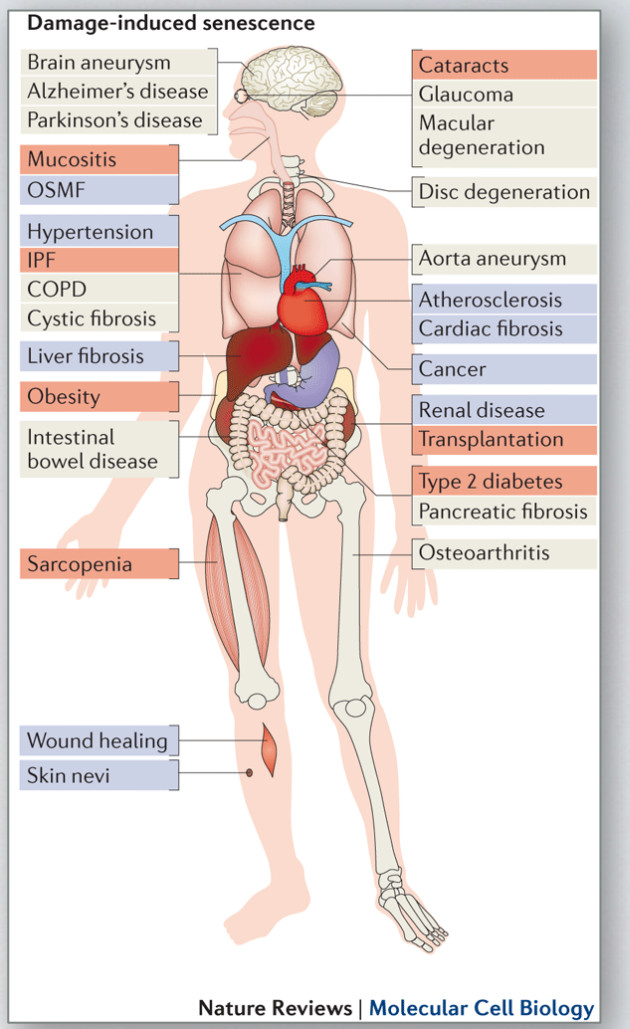
Senescent Cells and Aging

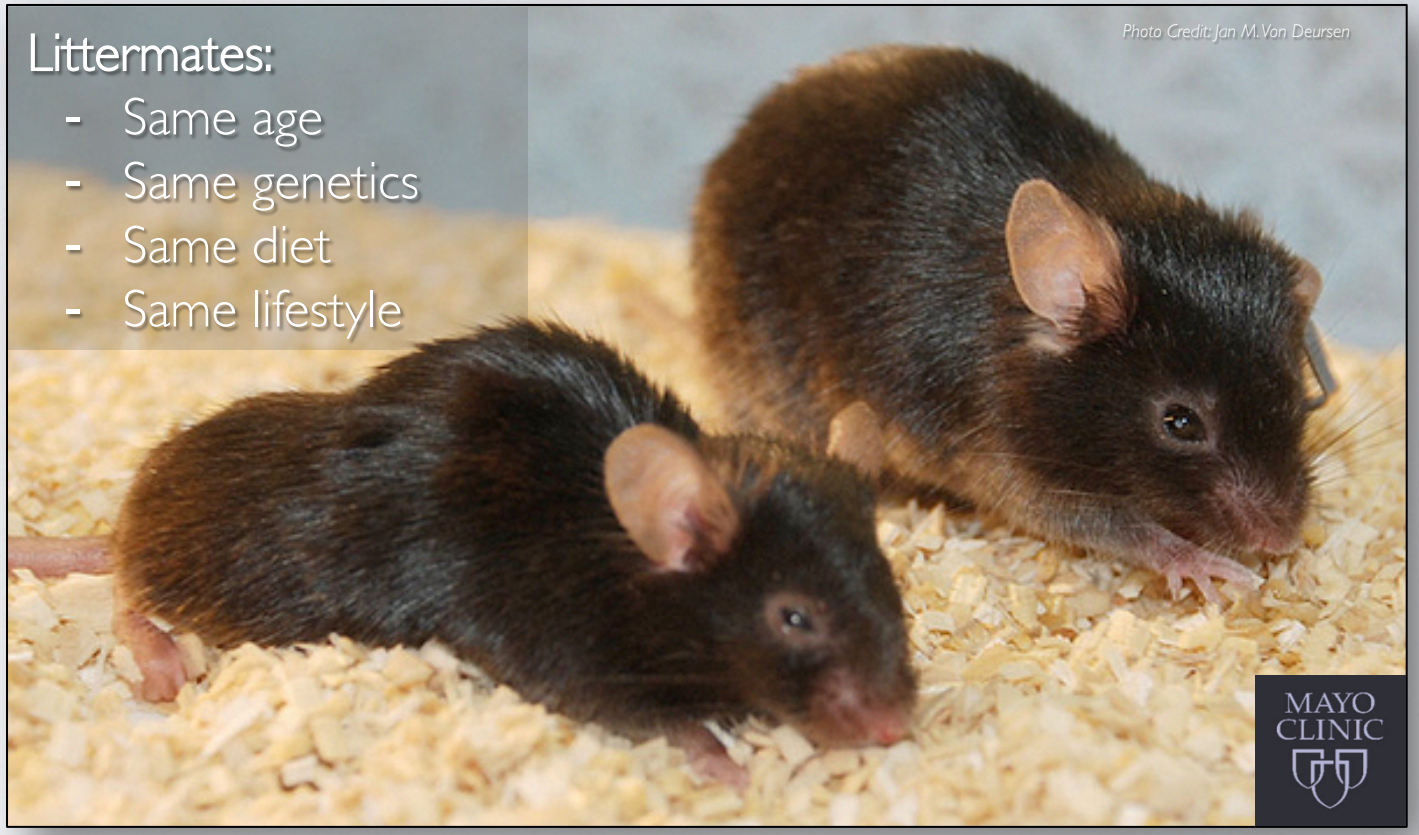


Cellular senescence: from physiology to pathology, Daniel Muñoz-Espín & Manuel Serrano, Nature Reviews Molecular Cell Biology 15, 482–496 (2014) doi:10.1038/nrm3823



Removing Senescent Cells Ameliorates Aging

- Mice were genetically engineered to drive an inducible suicide gene using the p16 promoter
- Suicide gene was induced using a chemical inducer of dimerization (AP20187), causing selective ablation of senescent cells
- Significant improvements in healthspan and lifespan



Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Baker et al., Nature 2016 Feb 11;530(7589):184-9

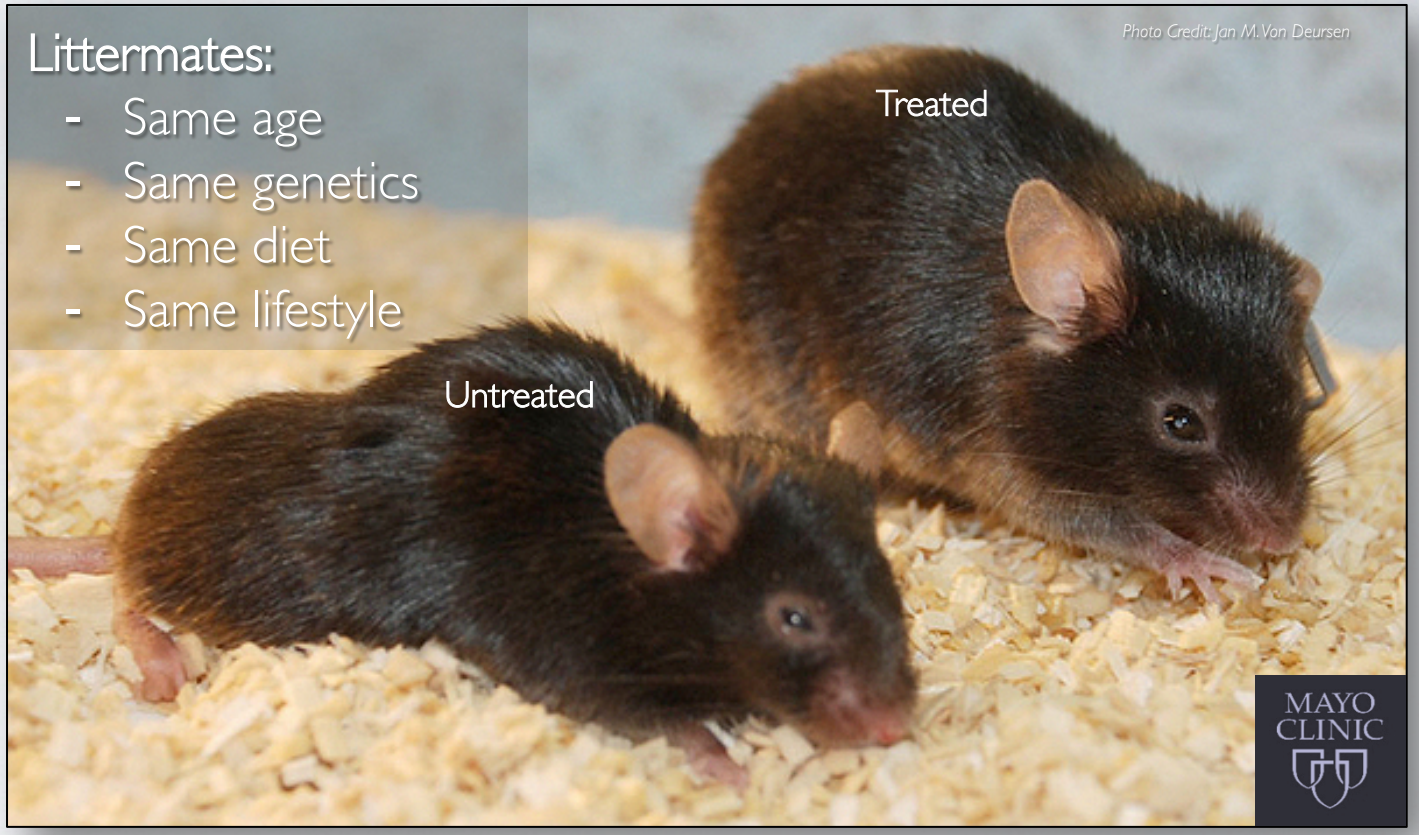
Removing Senescent Cells Ameliorates Aging

Treated:

- 25% median lifespan increase
- 50% less cancer

Untreated:

- Cataracts
- Frailty
- Loss of hair
- Cancer

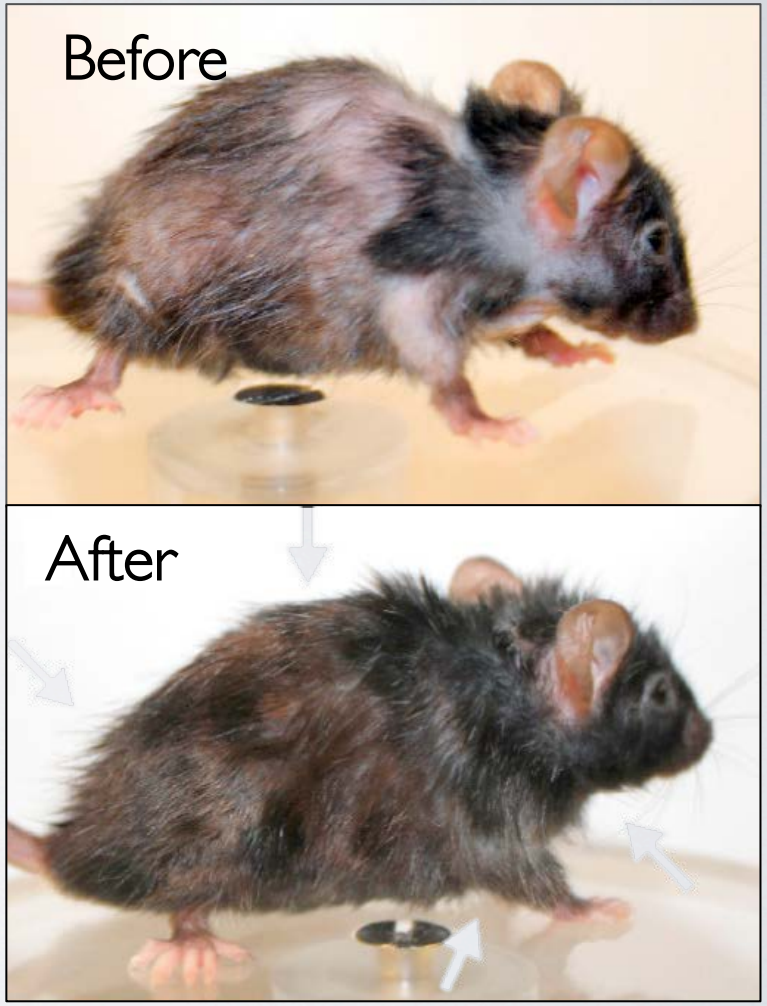


Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Baker et al., Nature 2016 Feb 11;530(7589):184-9

Clearing Senescent Cells Leads to Regeneration

- Senescent cells were ablated in fast- and naturally-aged mice
- Significant improvements in fitness, fur density, and renal function
- Amelioration of chemotherapy-induced toxicity

SAME AGED ANIMAL
trichothiodystrophy (TTD) model



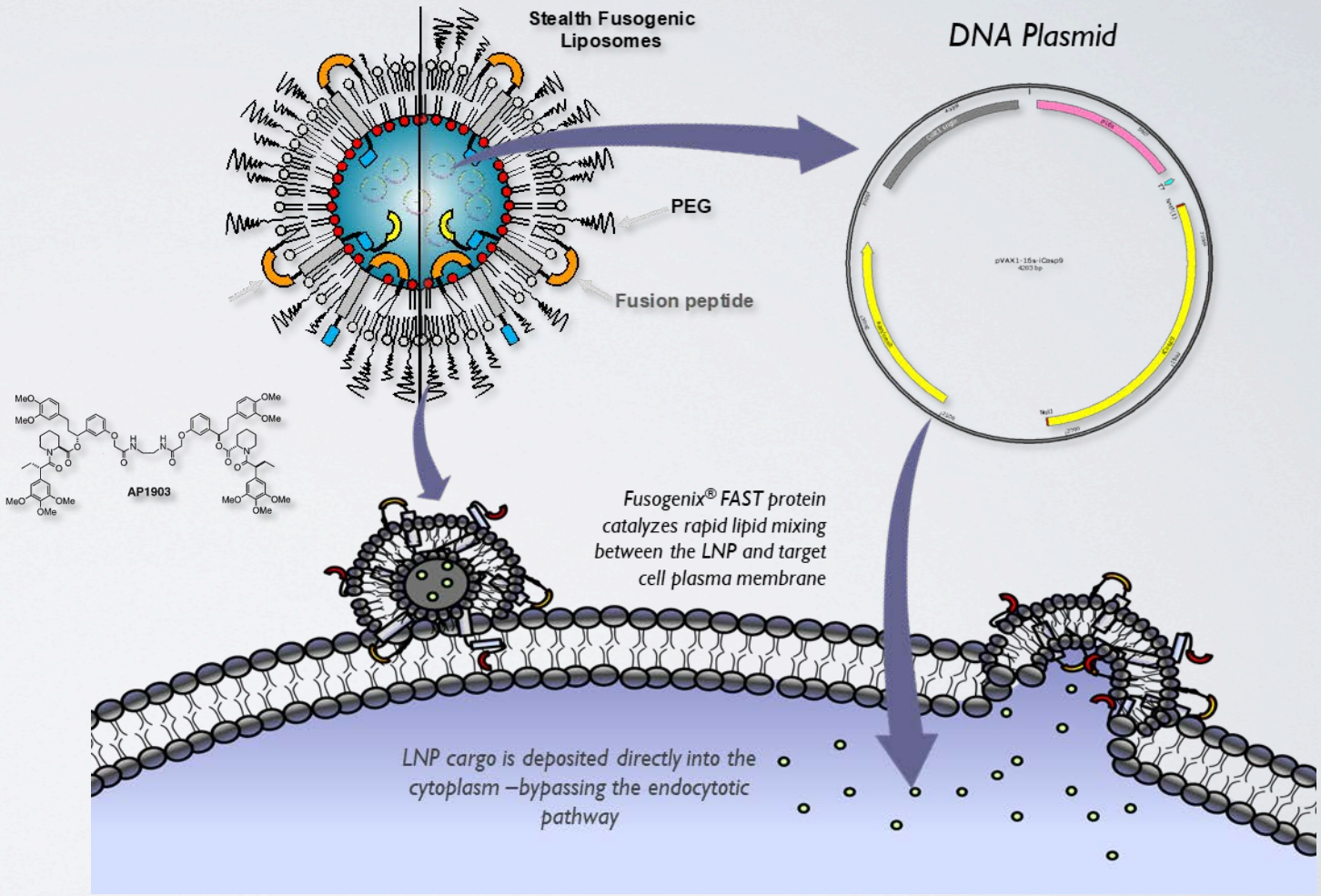
Adapted from Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging, Baar et al., 2017, Cell 169, 132–147

Ideal Senolytic Strategy: Considerations?

- Utilize strategy that is similar to successful *in vivo* approaches
- Well tolerated at therapeutic doses in healthy/aged individuals
- Suitable for repeated or prolonged dosing
- Targeting to specific tissues

Oisin's SENSOlytic™ Platform

- Non-integrating DNA plasmid, regulated by senescence promoter, driving a potent conditional suicide gene
- Chemical inducer of dimerization, to potentiate cell death
- Fusogenix lipid nanoparticle (LNP) delivery system, a non-toxic nanoparticle that uses a fusion protein to deliver plasmids directly into the cytoplasm

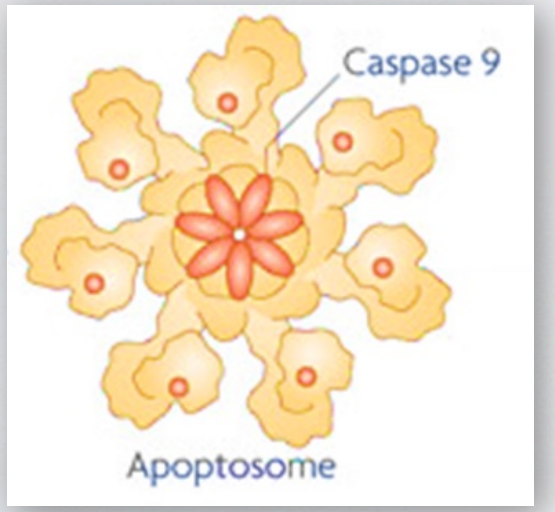


DNA Payload: A Highly Selective Suicide Gene

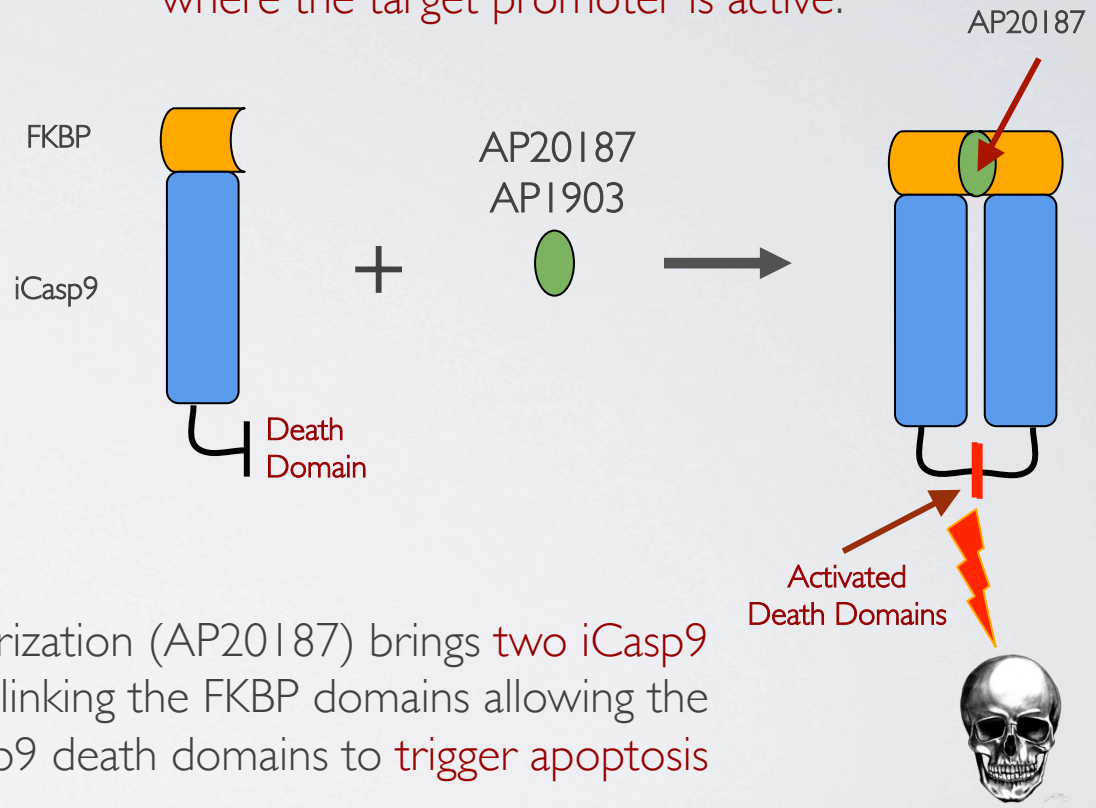
- DNA plasmids encode a death protein (caspase 9) under a promoter that is active in the target cell population.
- These DNA constructs are effectively logic gates written in DNA (IF/OR/AND) that allow us to precisely target cell populations based on gene activity without harming adjacent cells.
- Built a library of plasmids with different promoters of various strengths, linked to both inducible and self-activating caspase 9.

Suicide Gene: Inducible Caspase-9 (iCasp9)

- Based on endogenous caspase-9, functions late in apoptotic pathway
- Bypass any upstream abnormalities (eg. Bcl-2 overexpression)
- Apoptosis induction is independent of cell cycle



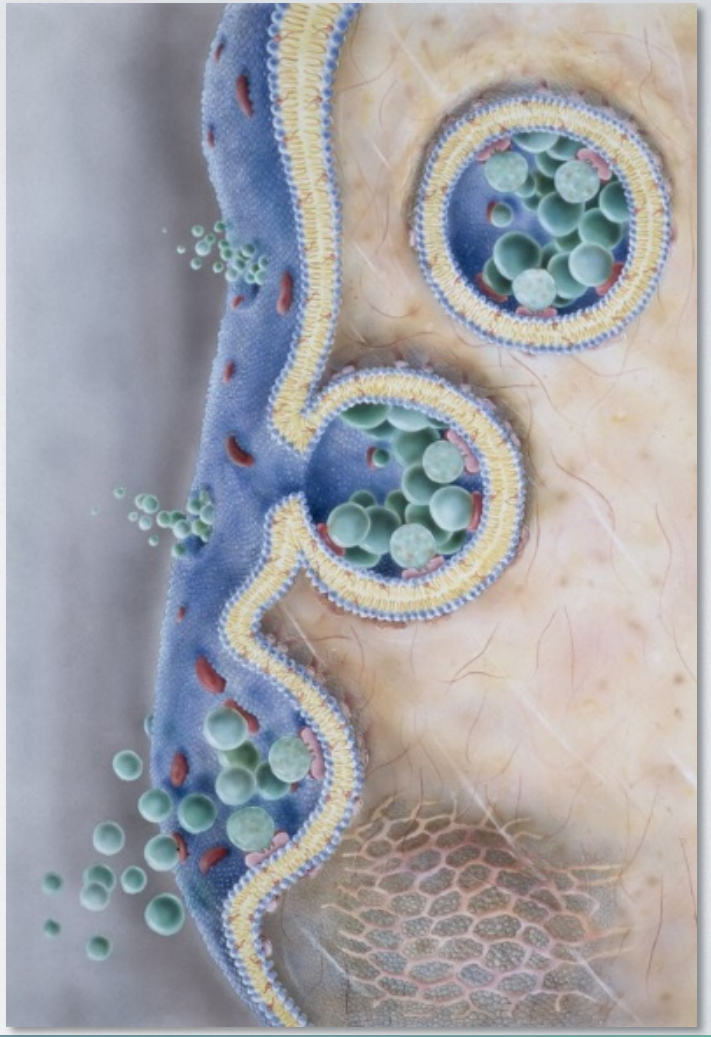
The suicide switch is produced only in cells where the target promoter is active.



Chemical Inducer of Dimerization (AP20187) brings two iCasp9 molecules together by linking the FKBP domains allowing the iCasp9 death domains to trigger apoptosis

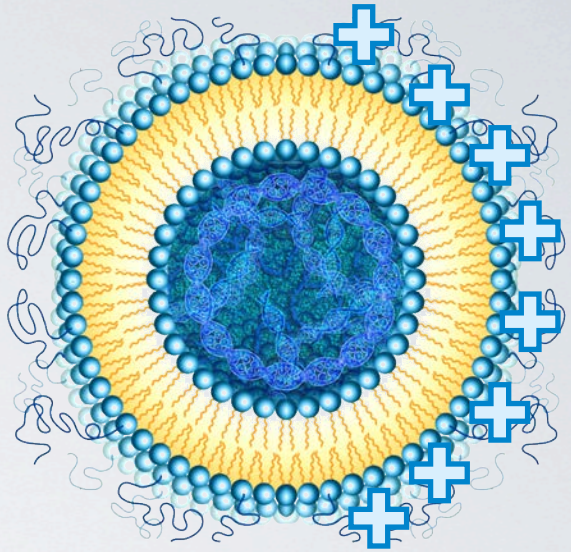
In Vivo Gene Delivery Is Challenging!

- Nucleic acid therapies need help to reach their targets
  - Without protection, the body's natural defences break them down
  - Rely on the bloodstream to get to their targets
- Getting to the outside the cell is not enough, must be intracellular for activity
- Cell membrane - A tough physical barrier
  - Actively repels foreign substances
  - Endo-lysosomal pathway degrades foreign particles
- Current drug delivery systems depend on the endocytic pathway
  - Natural defence mechanism that degrades cargo
  - Cargo must escape to become active



Lipid-based Delivery Systems

Lipid nanoparticles are an established platform for *in vivo* drug delivery



- Improved protection
- Improved pharmacodynamics, biodistribution
- Tunable circulation time and targeting

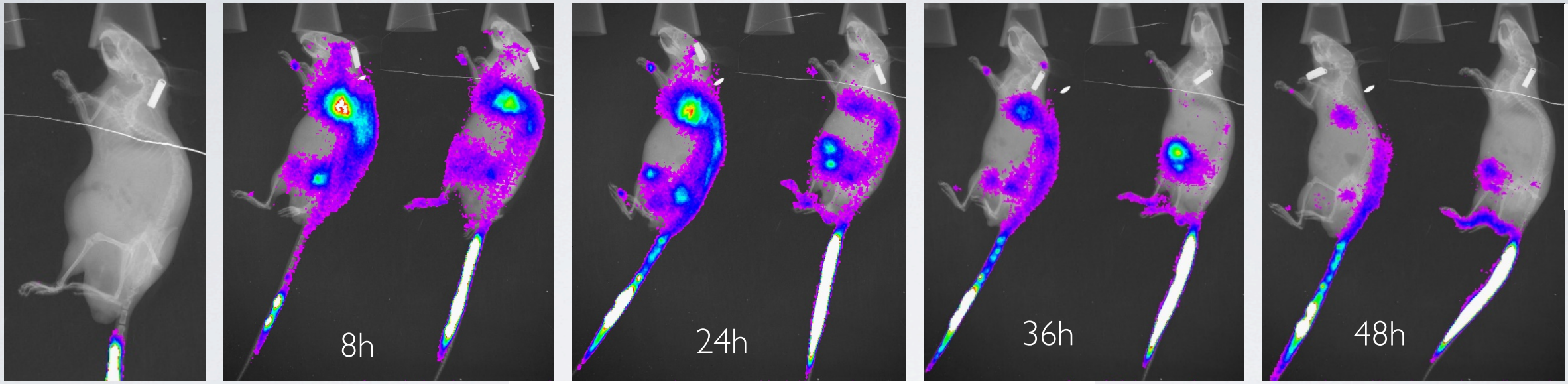
Intracellular delivery is dependent upon cationic lipids

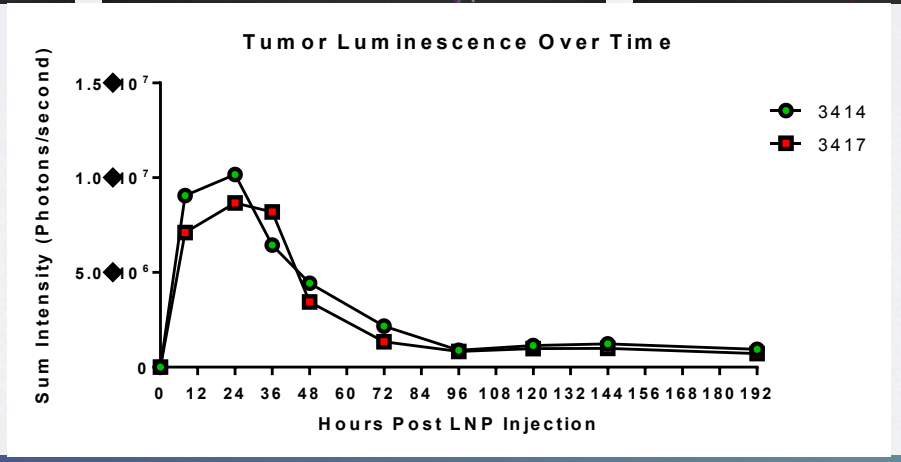
- Neutral liposomes (NL) have no charge, but are relatively poor at intracellular delivery
- Cationic liposomes (CL) have strong positive charge and deliver efficiently, but are highly toxic

Traditional LNPs Have Safety Issues

Comparison Of Maximum Tolerated Dose (MTD) Clinical Stage Lipid-based Delivery Technologies				
Company	Technology	Type	MTD	Clinical Development
Various	Lipotrust, DOTAP, others	Cationic Lipid (CL)	0.045 mg/kg	Phase I (various - all terminated due to toxicity)
Calando/Arrowhead	RONDEL lipopolymer	CL	0.81 mg/kg	Phase II (CALAA-01)
EGEN	PEG-PEI-Cholesterol Lipopolymer	CL	0.65 mg/kg	Phase II (EGEN-001, failed due to toxicity)
Marina Biotech, ProNAi	Smarticles (amphoteric liposomes)	Conditionally Cationic Lipid (CCL)	3.2 mg/kg	Phase II (PNT2258)
Marina Biotech	DiLA^2	CCL	1 mg/kg	Phase I
Arbutus (Tekmira), Alnylam	SNALP	CCL	0.9 mg/kg	7 programs in Phase I and II
MD Anderson (Anil Sood)	DOPC liposomes	Neutral Lipid (NL)	>10 mg/kg	Phase I (siRNA-DOPC-EphA2)

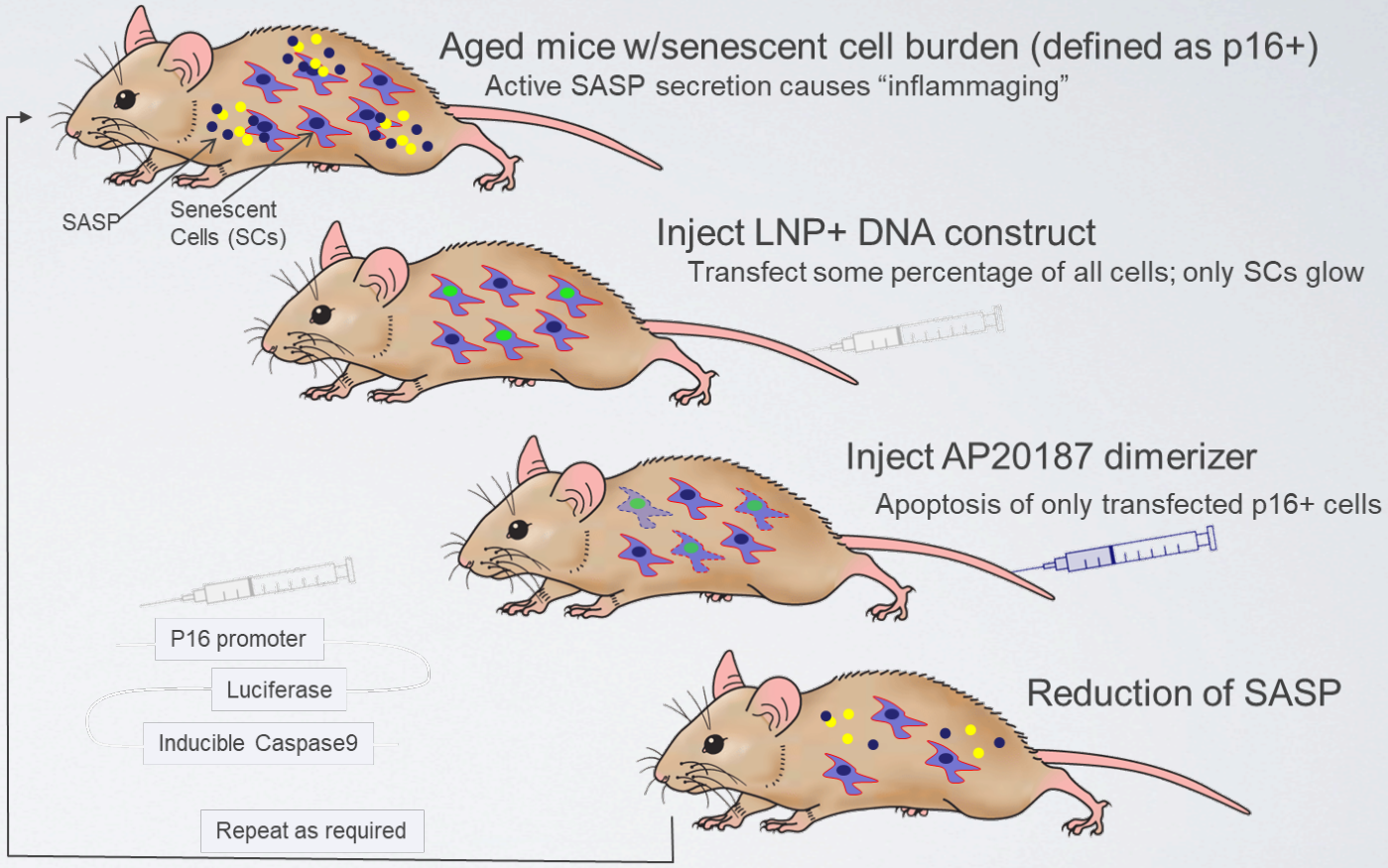
Systemic Nucleic Acid Delivery *In Vivo*





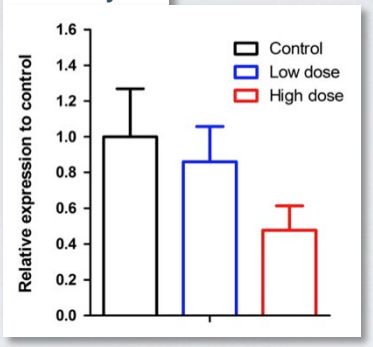
In Vivo Evaluation Of P16+ Senescent Cell Ablation

- 16 naturally aged C57BL/6 mice (all male), 80 weeks
- Control, Low dose (5 mg/kg) and High dose (10 mg/kg)
- Each animal received a single dose

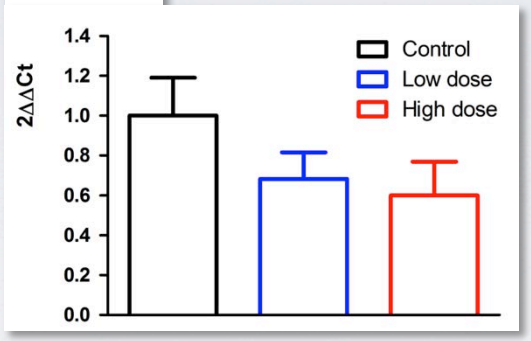


Dose-dependent Targeting Of p16-expressing Cells In Naturally Aged Mice

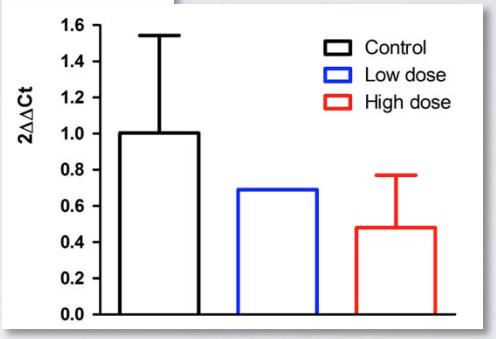
Kidney



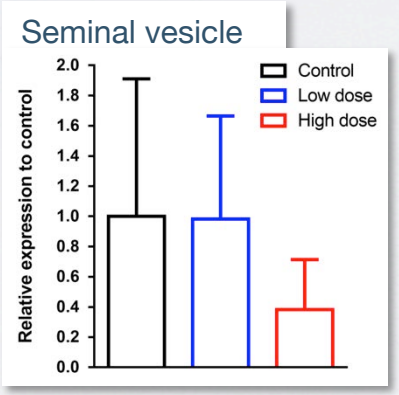
Spleen



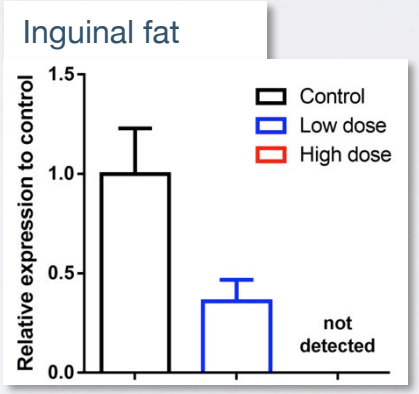
Lung



Seminal vesicle



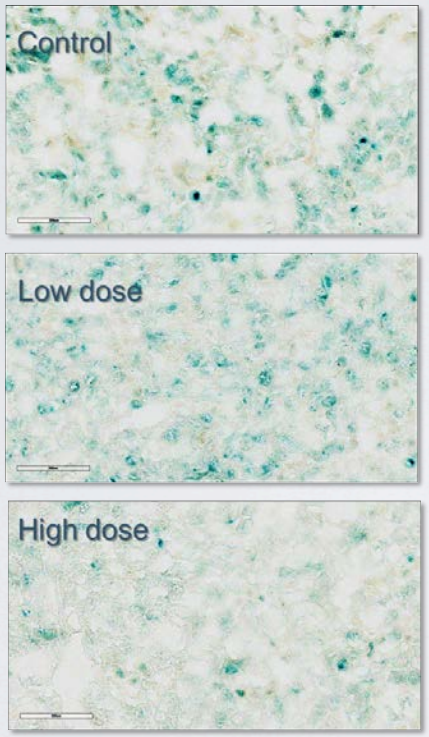
Inguinal fat

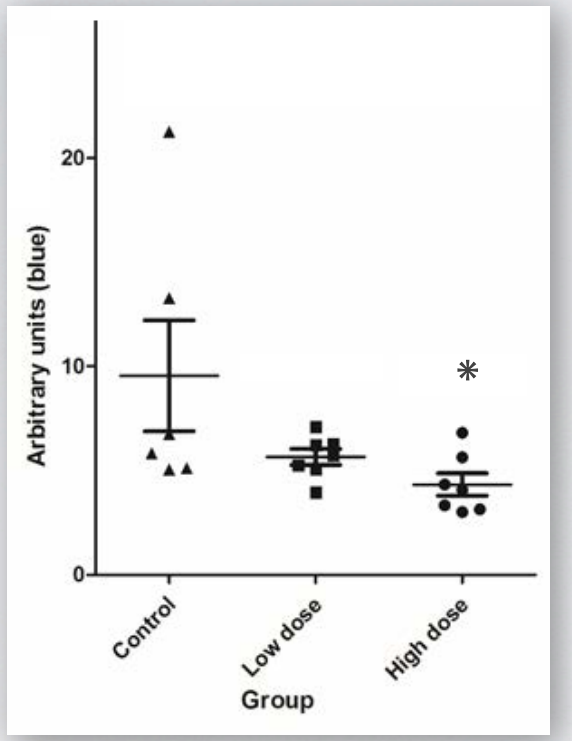


Tissues from mice treated with pVax-p16 Fusogenix formulations were subjected to qRT-PCR to detect $p16^{Ink4a}$

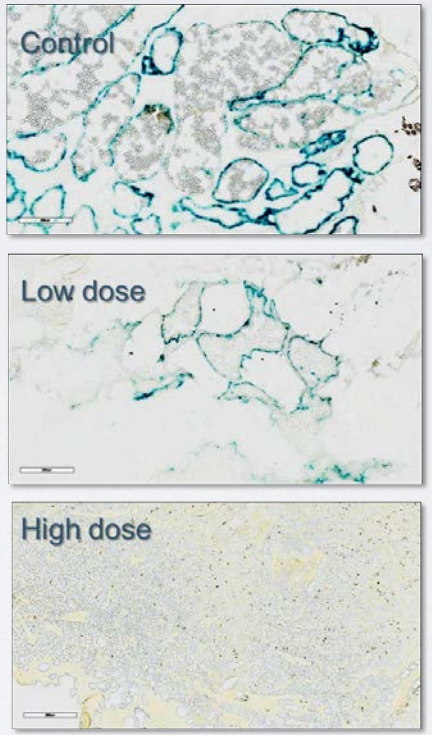
Dose-dependent Targeting Of p16-expressing Cells In Naturally Aged Mice

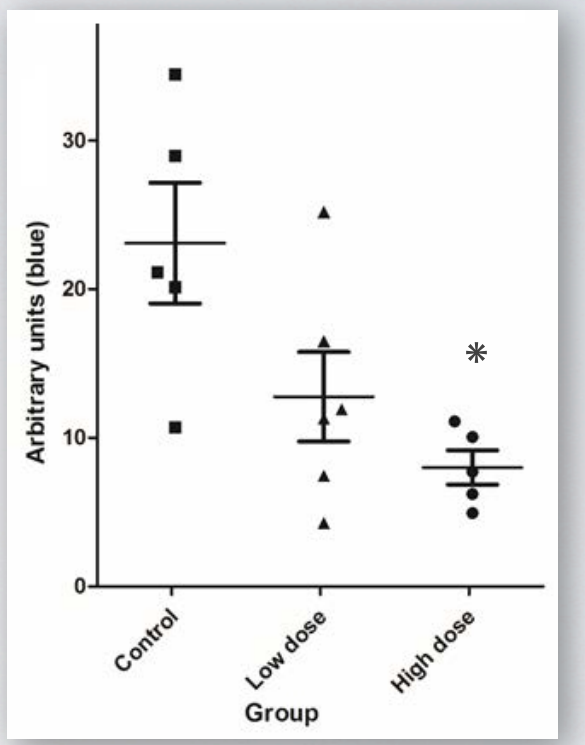
Kidney





Seminal Vesicle

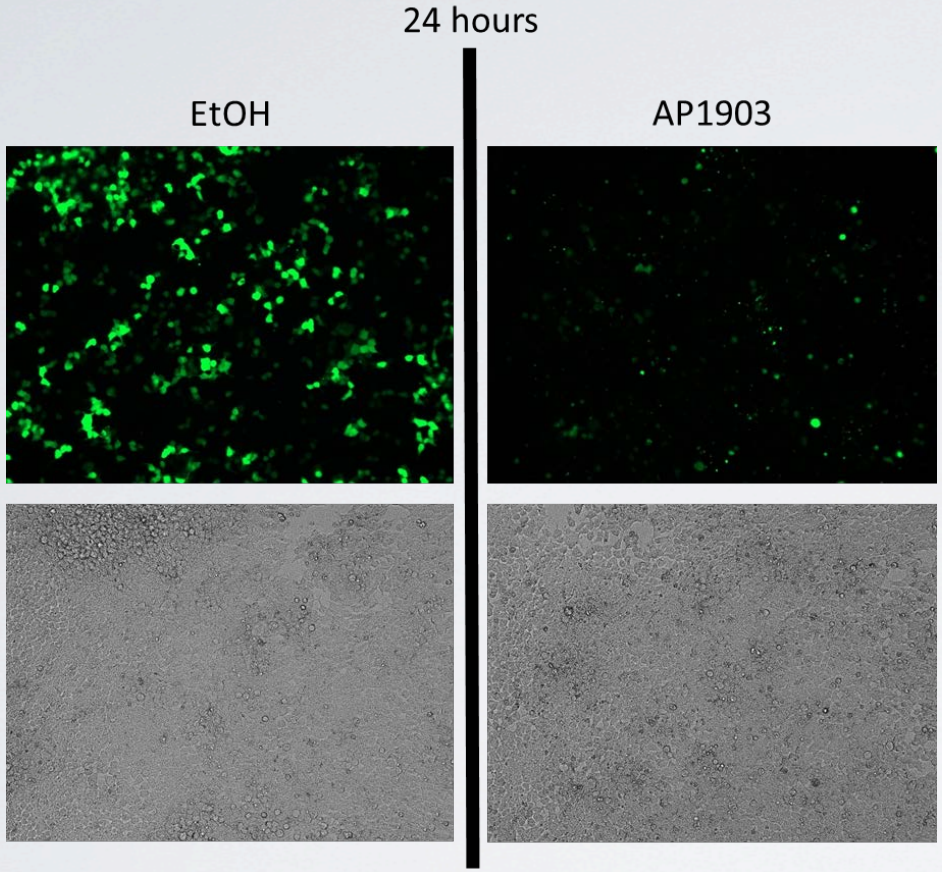


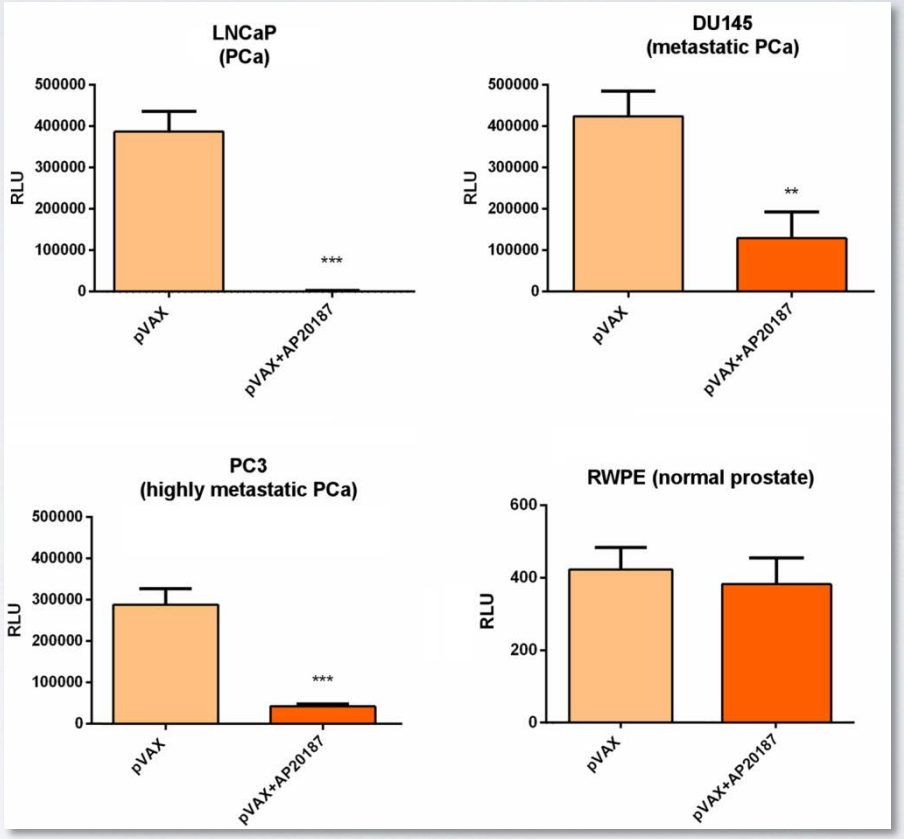


From Senescence To Cancer

- An iCasp9 plasmid driven by a p16 promoter selectively kills senescent cells through apoptosis
- SENSOlytic™ LNPs encapsulating this plasmid payload ablate senescent cells *in vivo* in a dose-dependent manner – could this work against cancer?

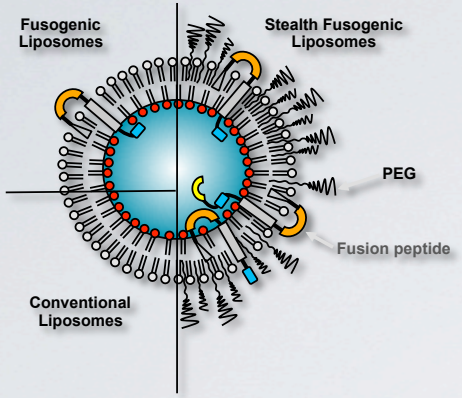
Can We Selectively Kill Prostate Cancer?

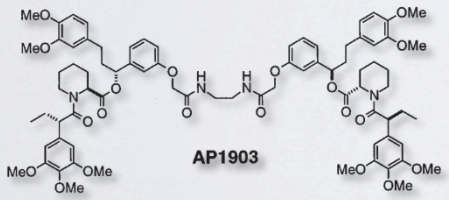




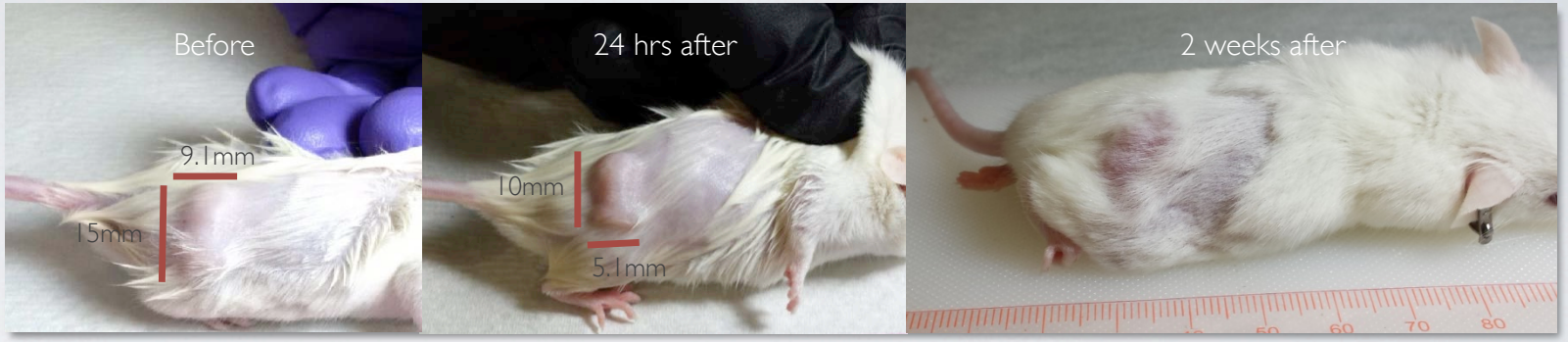
Human prostate cancer (LNCaP, DU145, PC-3) or normal epithelial (RWPE) cells were treated with Fusogenix lipid nanoparticles carrying the pVax-p53-iCasp9-luc plasmid and assessed for iCasp9 expression by Western blot and luminescence assays.

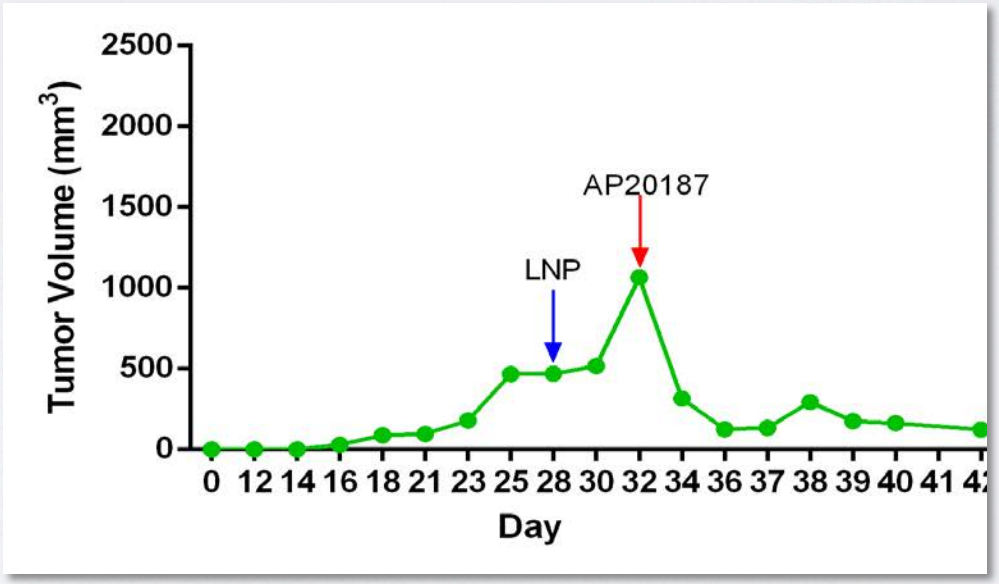
Suicide Gene Therapy Of Prostate Cancer





Single injection of LNP results in 75-90% reduction in tumor after 48 hours

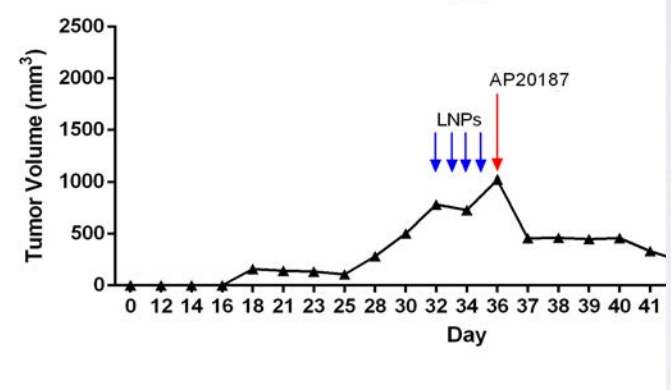


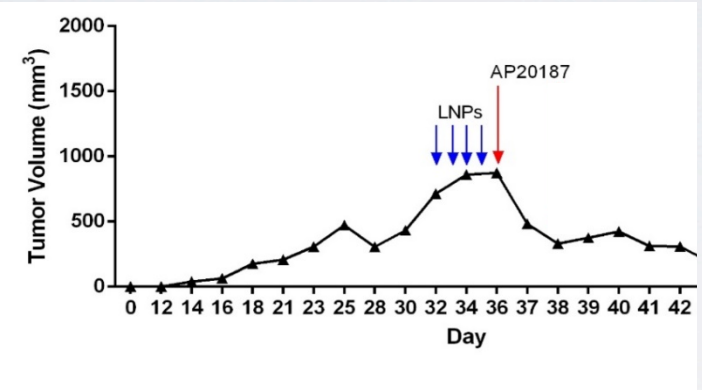


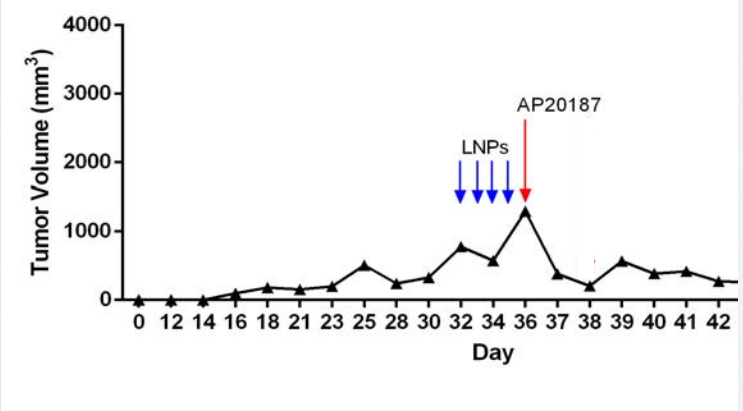
NSG mouse bearing a subcutaneous human prostate cancer PC-3 tumor was injected intratumorally with 100 µg Fusogenix pVax-p53 formulation, followed 96 hours later by 2 mg/kg AP20187 IV

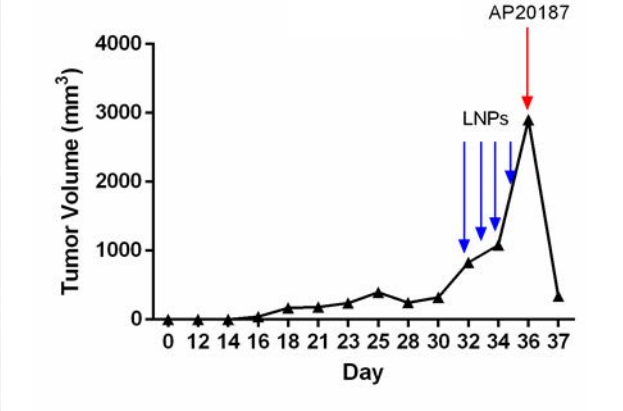
Suicide Gene Therapy Of Prostate Cancer

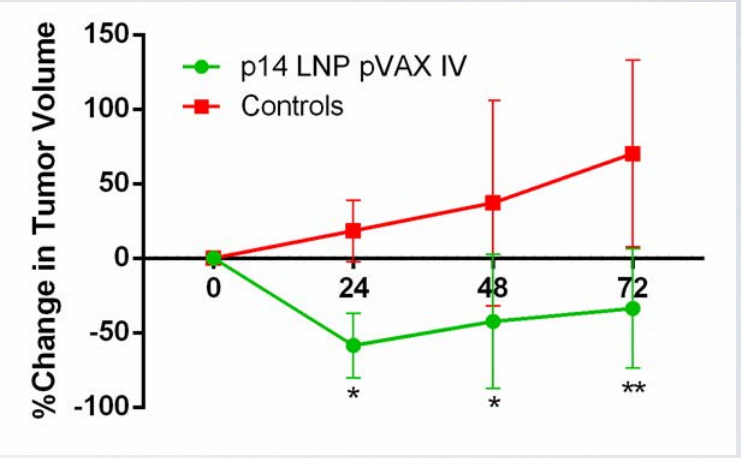
Single round of intravenous LNP treatment reduces prostate cancer tumors by 50-98% in 48 hours

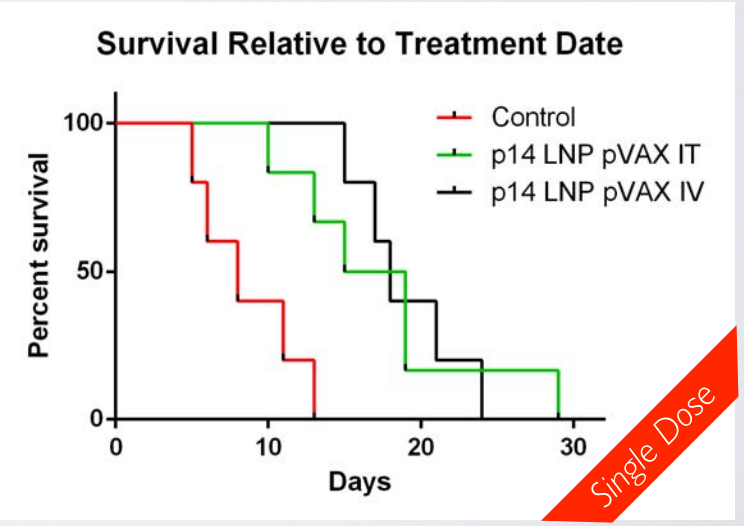






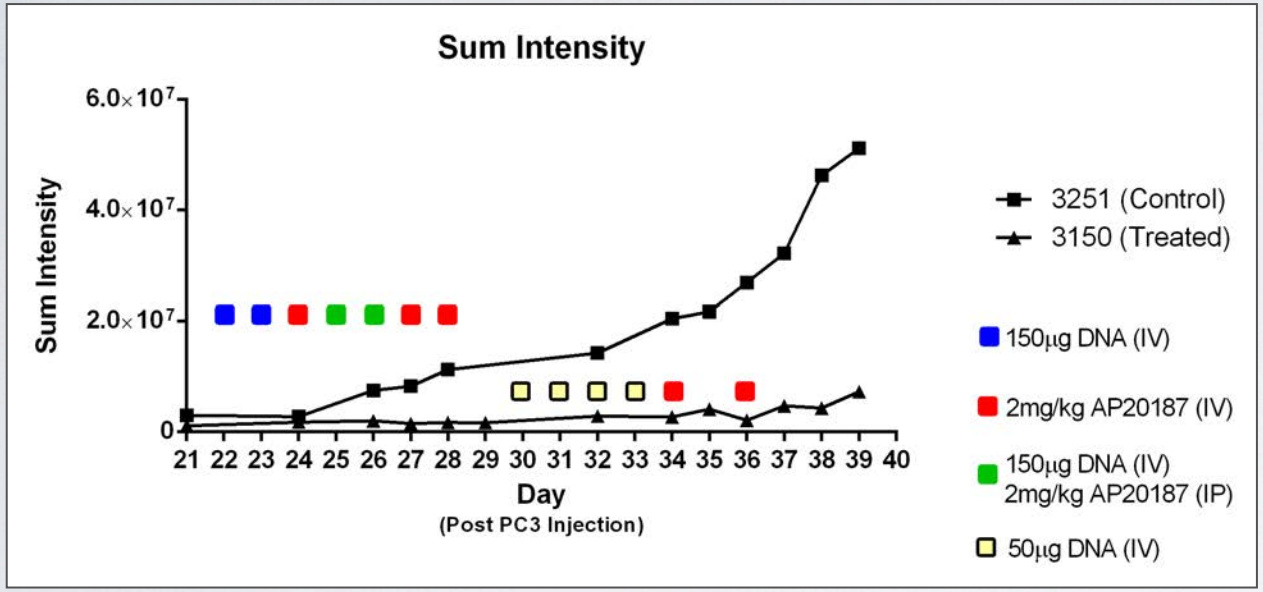






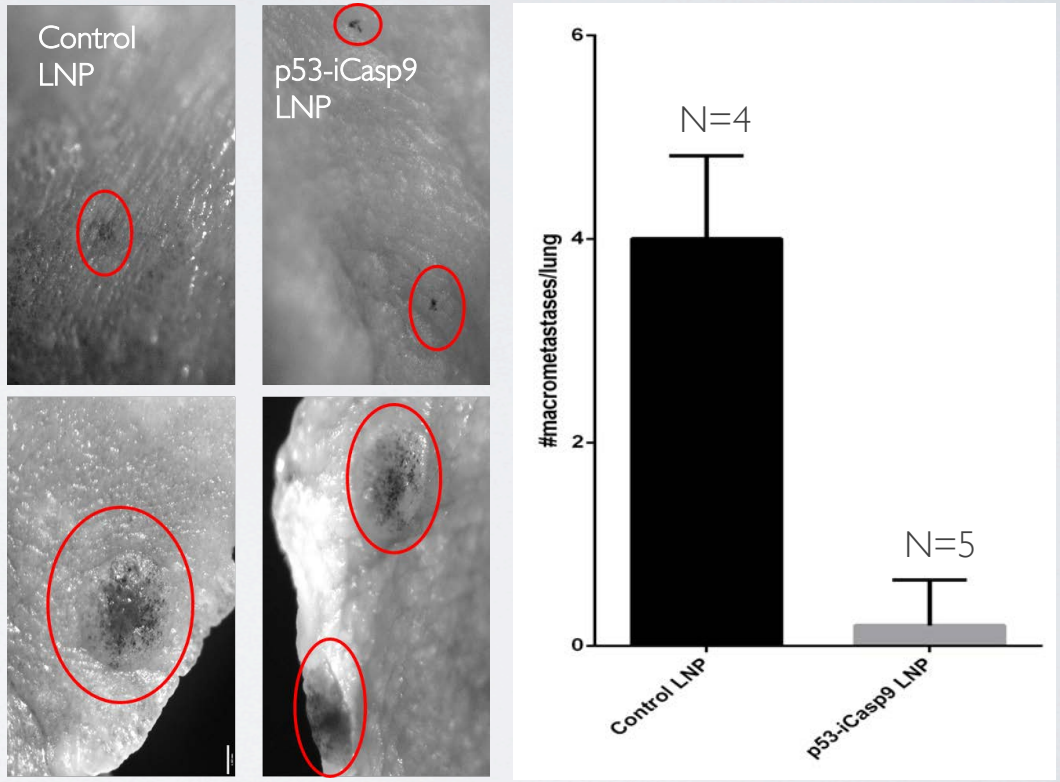
Control Of Metastatic Cancers

Human Prostate Cancer Metastases in NSG Mice
(Suppression of metastatic tumor with repeat treatment)



NOD-SCID mice were injected with 500,000 PC-3M-luciferase cells on Day 0, LNP dosing was started on Day 22 with 150µg p53-iCasp9 LNP. Dimerizer doses started Day 24 at 2 mg/kg. Mice were imaged every 24-48 hours to detect whole animal luminescence.

Mouse Melanoma Lung Metastasis
(immunocompetent)



LNP Safety and Biodistribution

TOXICOLOGY STUDIES

Anti-p14 / LNP antibody & neutralization assays	▰ No antibody response in immune-competent mice, even at high doses ▰ Neutralization requires very high ADA concentration ▰ "Vaccination" against p14/LNP doesn't reduce efficacy ▰ Repeat dosing is effective and well tolerated
CARPA Assays	▰ Fusogenix LNP is generally less reactive than Doxil
Non-Human Primate Studies	▰ *Chlorocebus sabaeus* (African green monkey), 14 animals. No toxicity seen in animals receiving both LNP and dimerizer at ten times normal human dose.

LNP Safety

Comparison Of Maximum Tolerated Dose (MTD) Clinical Stage Lipid-based Delivery Technologies				
Company	Technology	Type	MTD	Clinical Development
Various	Lipotrust, DOTAP, others	Cationic Lipid (CL)	0.045 mg/kg	Phase I (various - all terminated due to toxicity)
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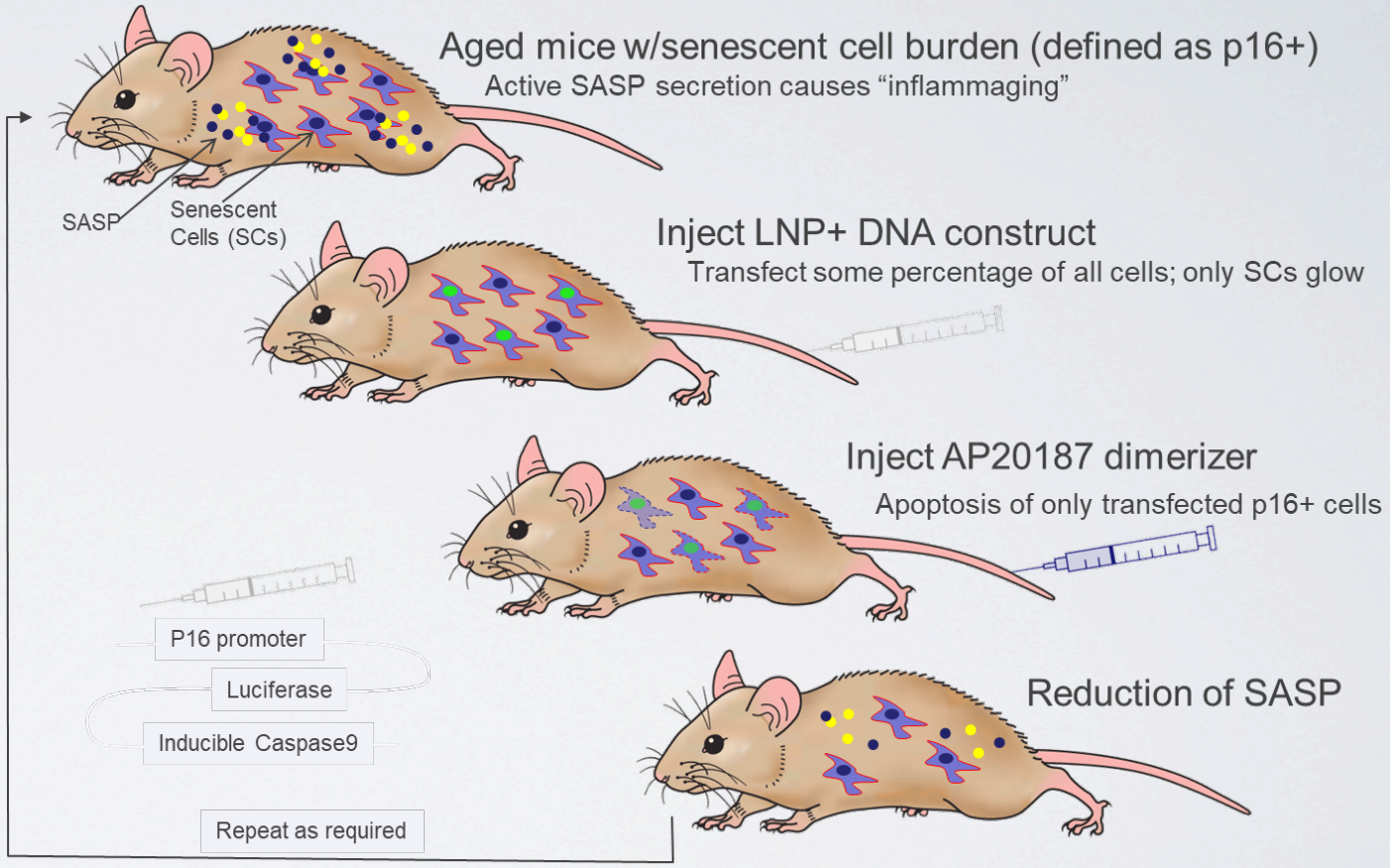
Marina Biotech, ProNAi	Smarticles (amphoteric liposomes)	Conditionally Cationic Lipid (CCL)	3.2 mg/kg	Phase II (PNT2258)
Marina Biotech	DiLA^2	CCL	1 mg/kg	Phase I
Arbutus (Tekmira), Alnylam	SNALP	CCL	0.9 mg/kg	7 programs in Phase I and II
MD Anderson (Anil Sood)	DOPC liposomes	Neutral Lipid (NL)	>10 mg/kg	Phase I (siRNA-DOPC-EphA2)
Oisín Biotechnologies	Fusogenix® Lipid Nanoparticles (LNPs)	Neutral Lipid (NL)	>15 mg/kg	Pre-clinical toxicology data in rats and NHP

Lifespan Study of Naturally Aged Mice
(p16, p53 and Combination Treatment)

- 40 aged C57BL/6 mice (M & F), randomized at 106 weeks
- Control, p16, p53 and combination
- Dosed once per month at 4 mg/kg until death
- INTERIM DATA after 6 months of treatments



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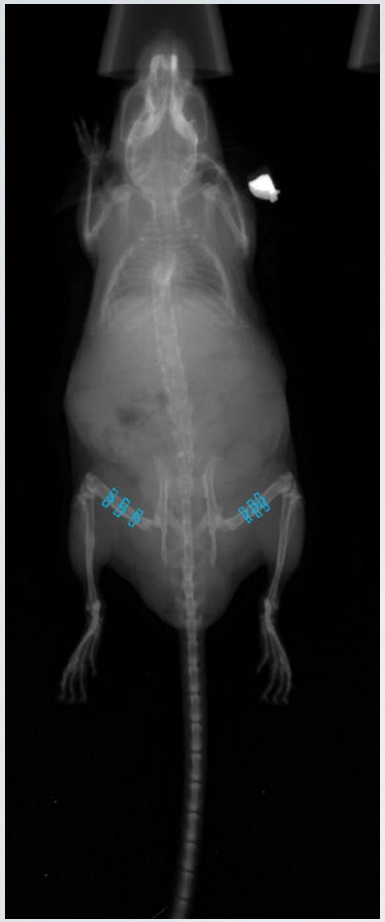
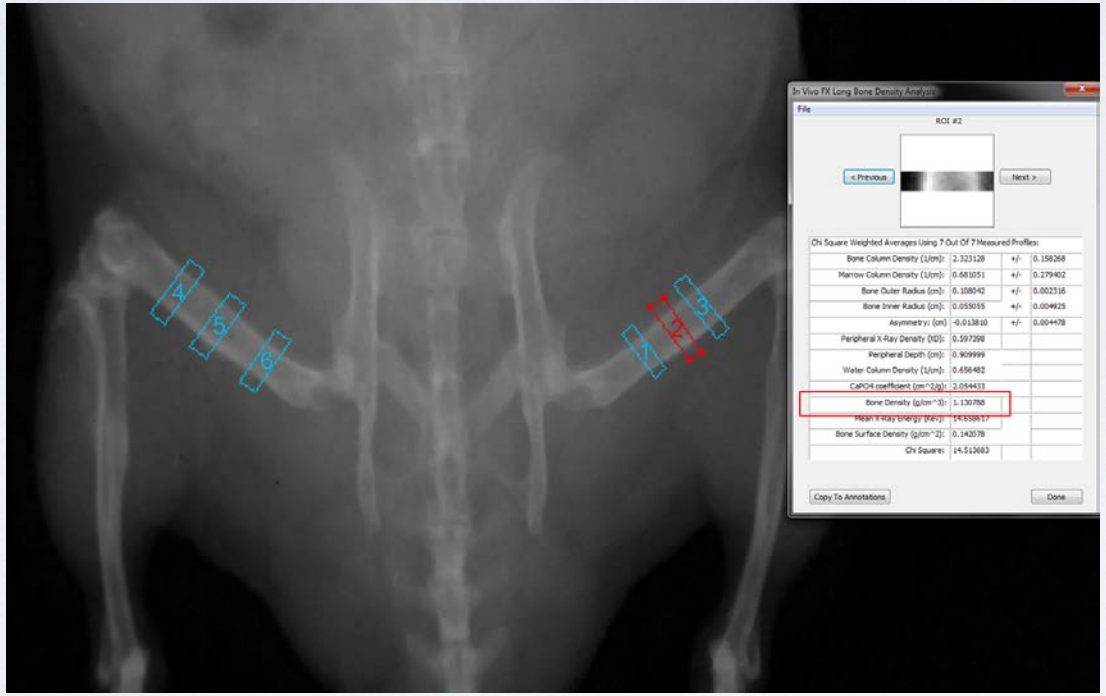
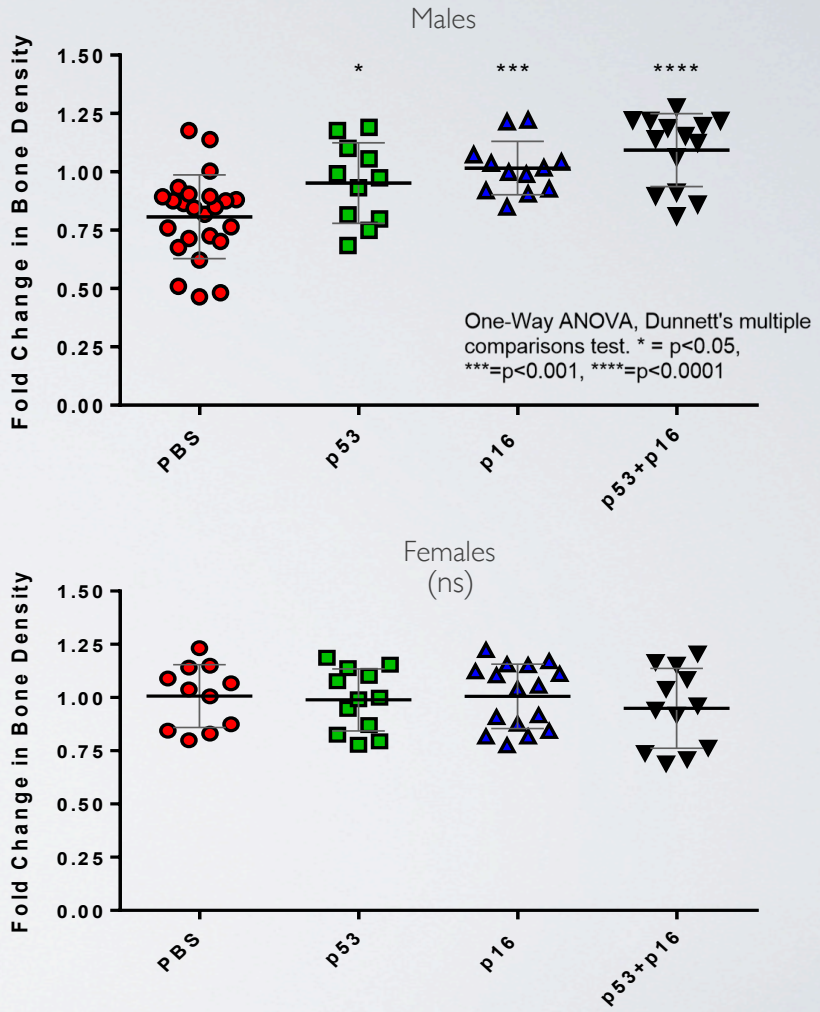
(p16, p53 and Combination Treatment)

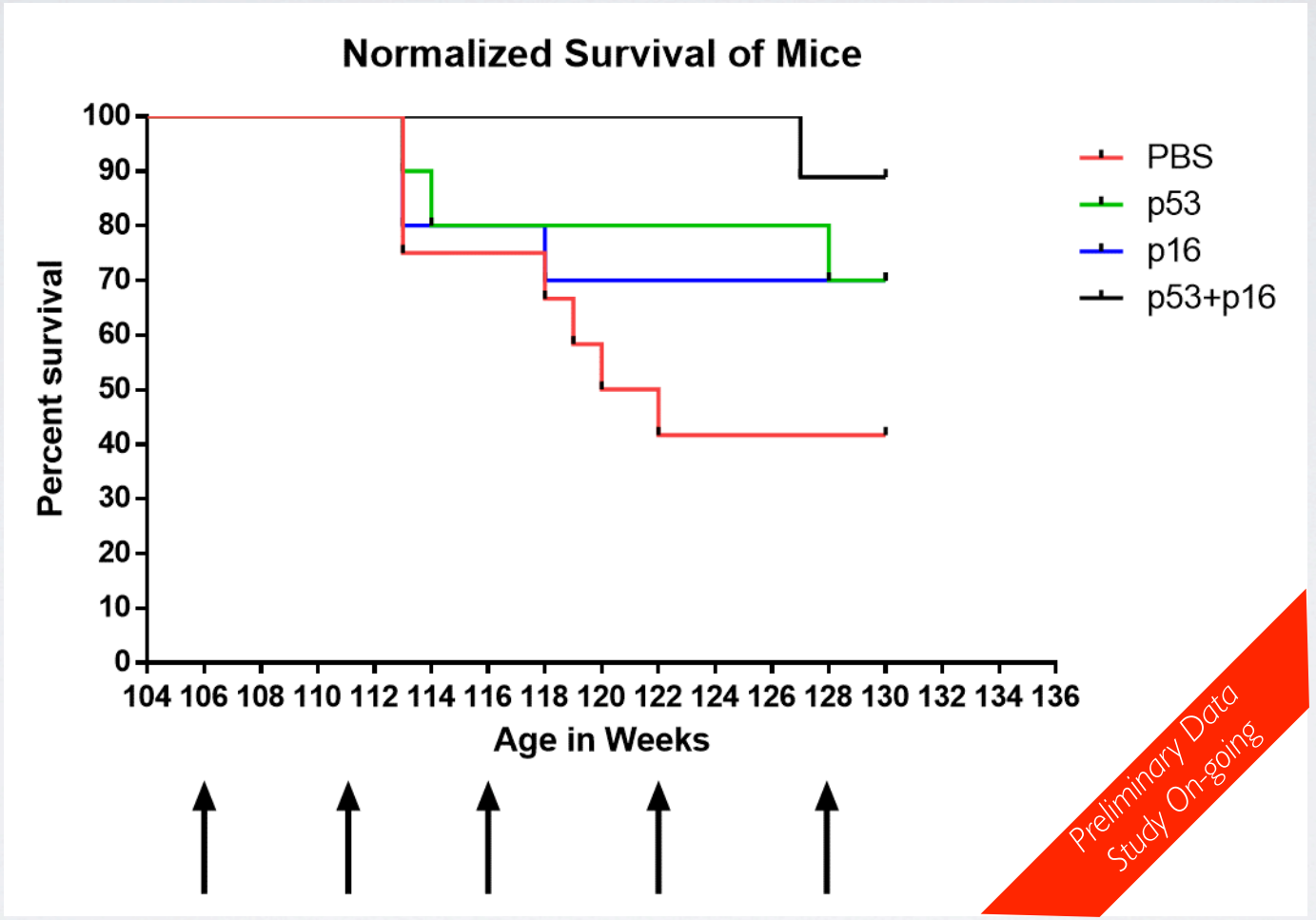


DEXA scans performed monthly





Lifespan Study of Naturally Aged Mice
(p16, p53 and Combination Treatment)



Summary And On-going Efforts

- Oisin's SENSOlytic approach is effective at ablating cells *in vivo* based on their transcriptional activation pathways
- First clinical candidate will be an oncology-targeted gene therapy
- Building a pipeline of senescence-based candidates and prioritizing therapeutic areas
- Evaluating biomarkers for aging, age-related degeneration

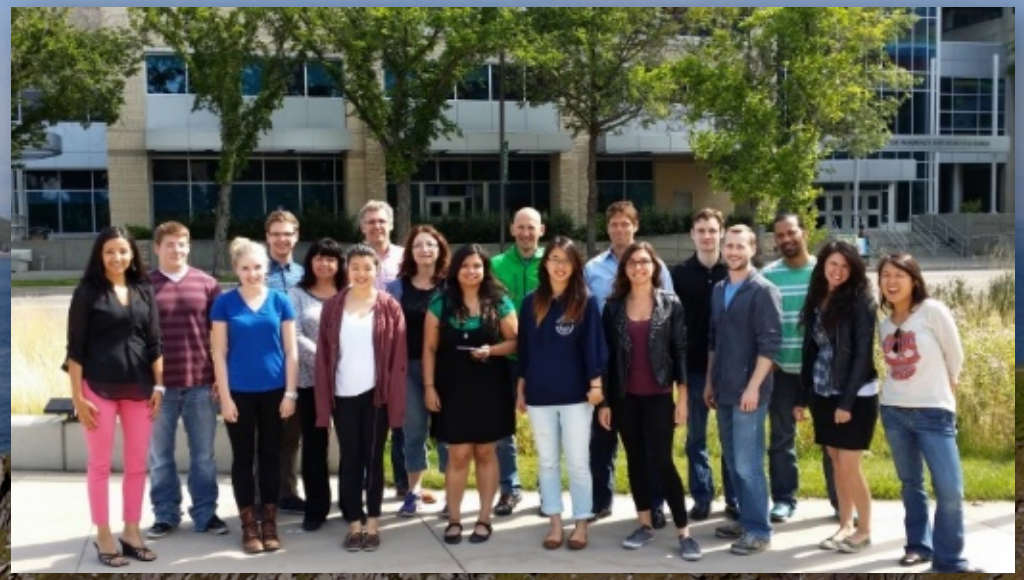
Thank You!


Gary C Hudson


Matthew Scholz


Steve Hilbert

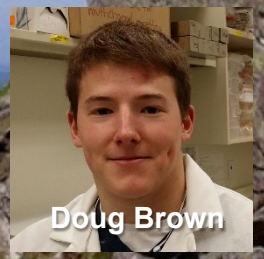

Kevin Perrott

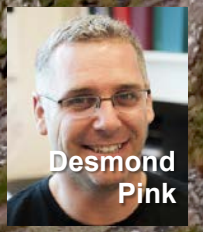



Andries Zijlstra


Roy Duncan


Arun Raturi


Doug Brown


Desmond Pink


Deborah Sosnowski

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