Mesenchymal stem cells engineered with TAT peptide functionalized nanoparticle Increase Therapeutic Efficacy of Anticancer Drug Through True Active Tumor Targeting

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Lung Cancer: Statistics

- 1 out of 4 cancer deaths is from lung cancer

- Lung cancer is the leading cancer killer in men & women in EVERY ETHNIC GROUP

- Worldwide lung cancer incidents are on track to increase by 38% to 2.89 million by 2030

- Lung cancer mortality is projected to reach 2.45 million worldwide by 2030, a 39% increase since 2018

- Average 5-year survival = 18%

- Require more effective treatment strategy
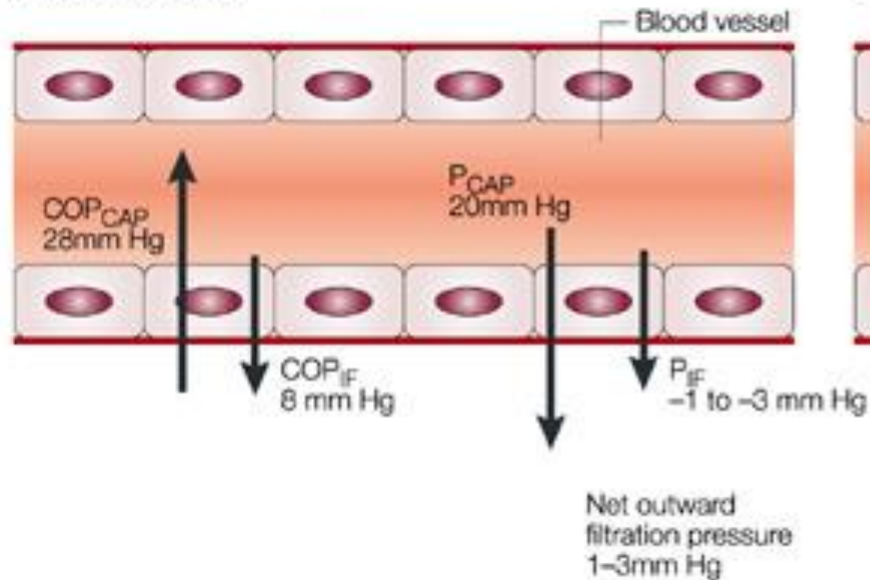
Cancer Therapy: Limitations…

- Poor availability of chemotherapeutics in deep-seated and metastatic cancers

- Development of drug resistance

- Dose-dependent cytotoxicity

- Need for targeted drug delivery to the tumor tissue

  - Improved therapeutic efficacy

  - Minimal toxic side effects
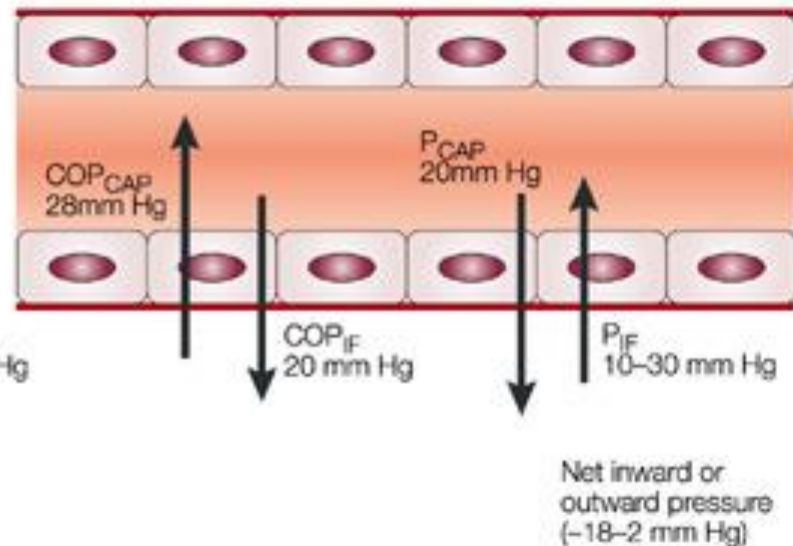
Enhanced Permeation and Retention Effect

Bisht and Maitra Nanomedicine and Nanobiotechnology 2009
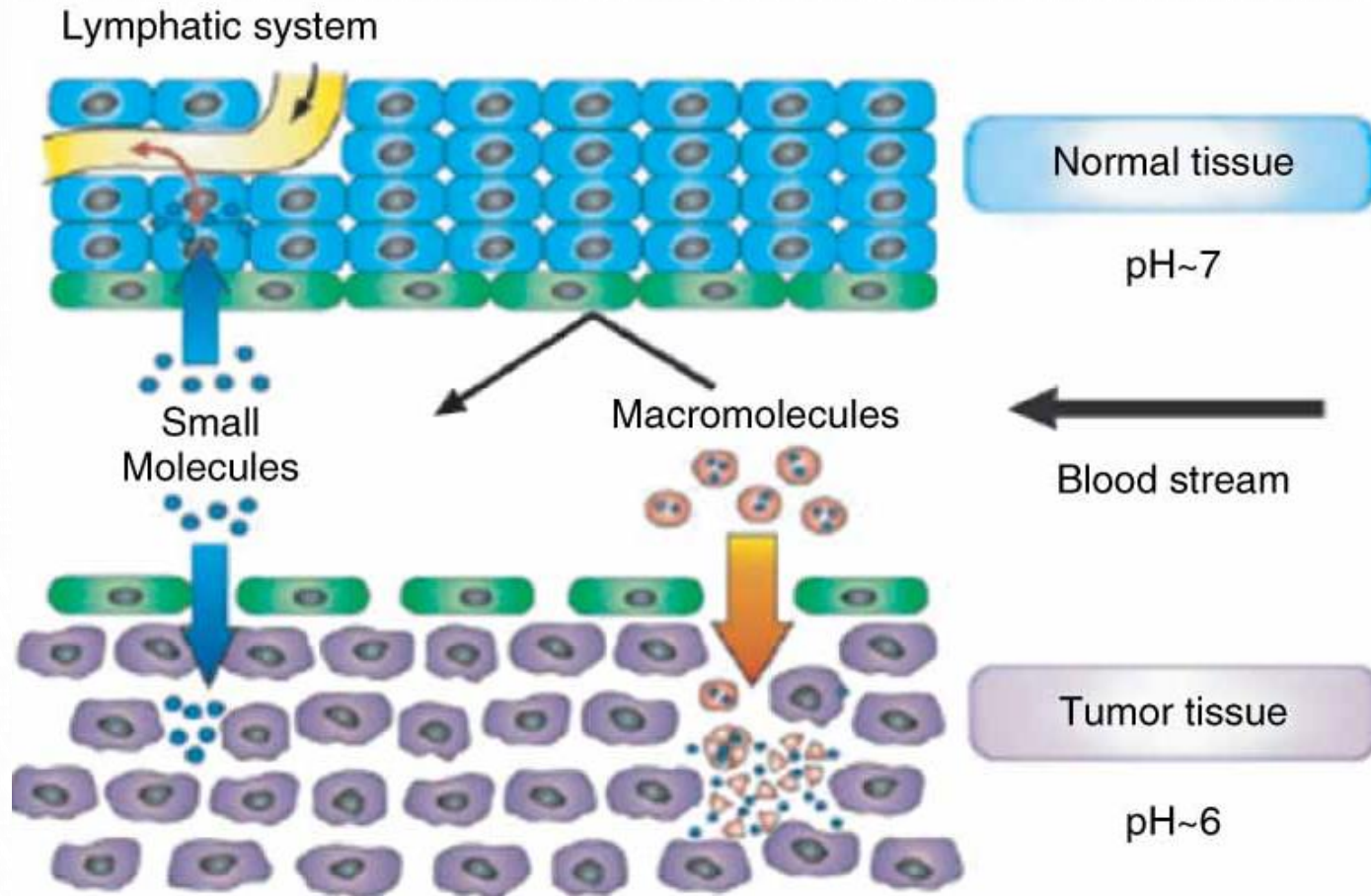
Elevated Interstitial Fluid Pressure Provides Resistance to Transport

a Normal tissue

COP$_{CAP}$ 28mm Hg

P$_{CAP}$ 20mm Hg

Blood vessel

COP$_{IF}$ 8 mm Hg

P$_{IF}$ −1 to −3 mm Hg

Net outward filtration pressure 1–3mm Hg

b Tumour tissue

COP$_{CAP}$ 28mm Hg

P$_{CAP}$ 20mm Hg

COP$_{IF}$ 20 mm Hg

P$_{IF}$ 10–30 mm Hg

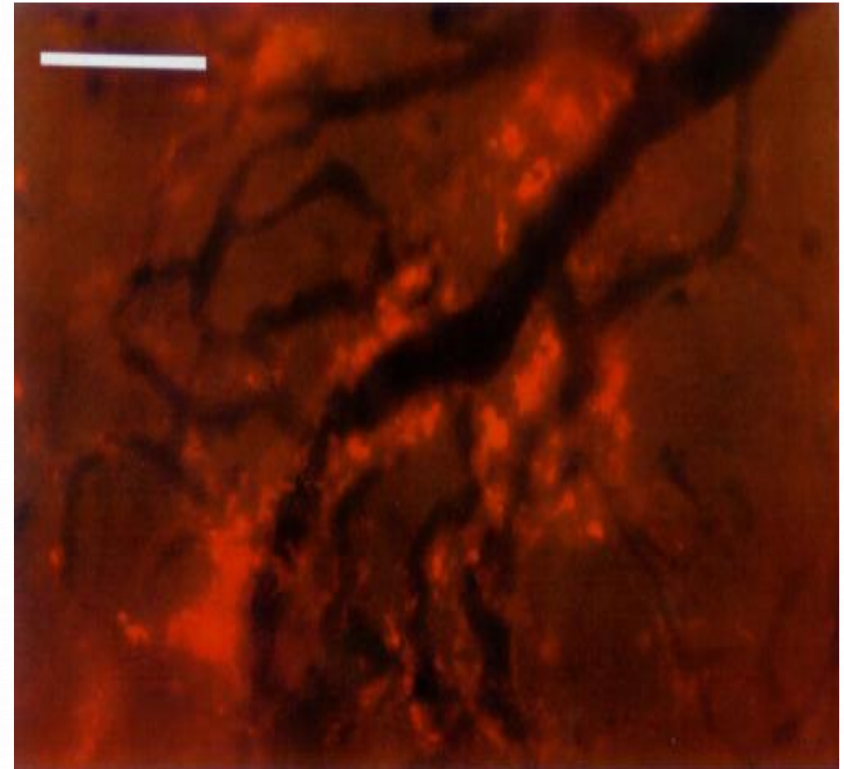Net inward or outward pressure (−18–2 mm Hg)

P_{CAP} and COP_{CAP} : hydrostatic and colloid osmotic pressures in capillaries
P_{IF} and COP_{IF}: hydrostatic and colloid osmotic pressures in surrounding inters

Heldin et. al. Nature Reviews Cancer **4**, 806-813 (2004)
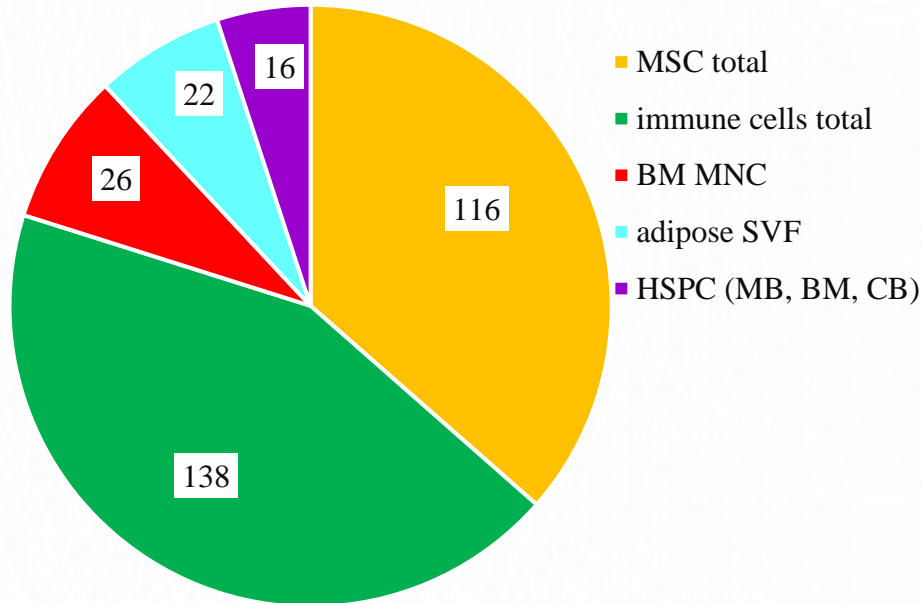
Spatial Heterogeneity in Permeability
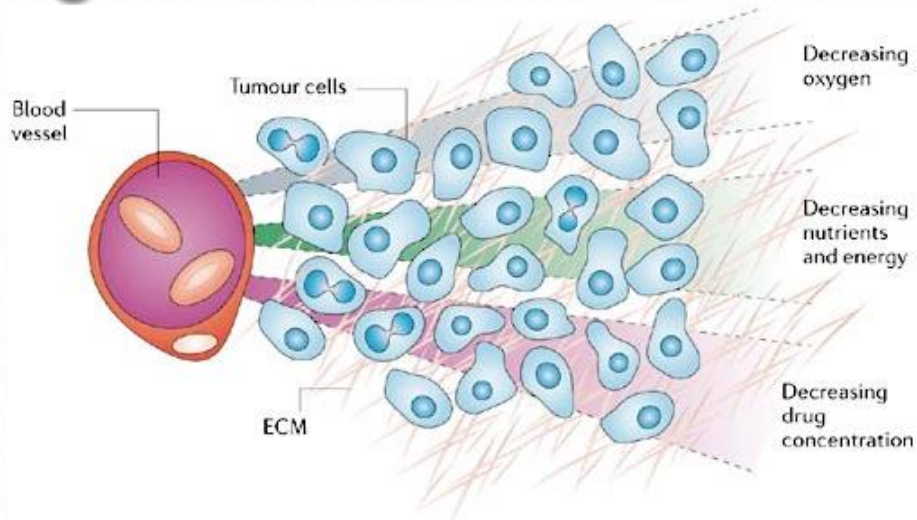
Cancer Research 54 (1994): 3352-3356

Cell Based Therapy

- Total 372 clinical trials have been registered

Major cell types (85% of all trials)

- MSC total
- immune cells total
- BM MNC
- adipose SVF
- HSPC (MB, BM, CB)

Indication

- cancer (123)
- muskuloskeletal (51)
- skin diseases (17)
- eye diseases (15)
- pulmonary (7)
- diabetes (5)
- neurology (48)
- cardiovascular (25)
- liver diseases (8)
- infertility (8)
- viral infection (19)
- others (46)
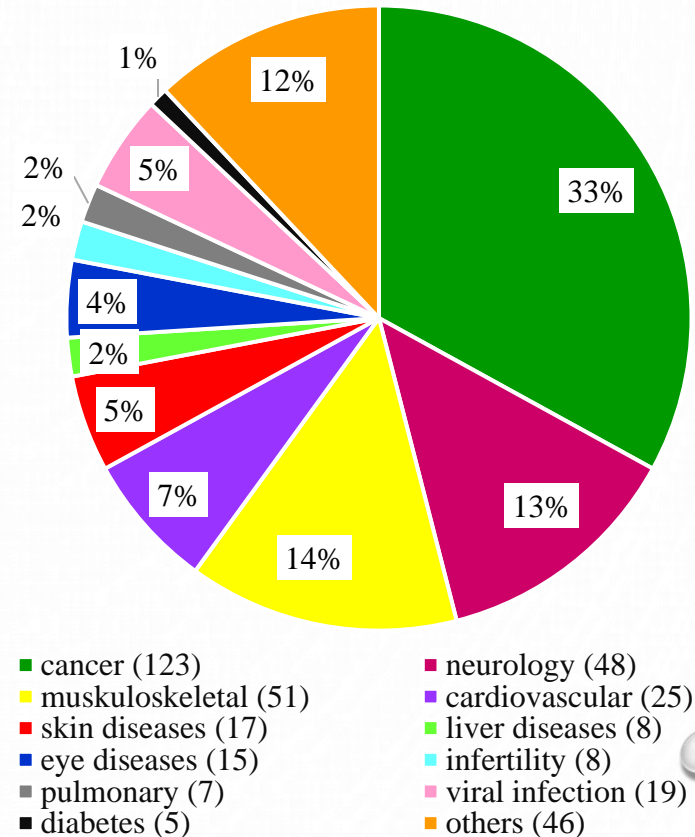
Bersenev Alexey. Cell therapy clinical trials – 2014 report. CellTrials blog. January 22, 2015. Available: http://celltrials.info/2015/01/22/2014-report/

What Are Mesenchymal Stem Cells (MSCs) ?

- First isolated by Friedenstein in 1970s

- Fibroblast like cells - spindle-shaped

- Adherent to tissue culture plastic

- High growth potential

- Surface markers: CD29, CD44, CD45, CD51, CD73 CD90/Thy-1, CD105, CD166, Integrin α1, PDGF, STRO-1, VCAM-1, IL-IR and absence of CD45, CD34, CD14, CD19, and HLA-DR.
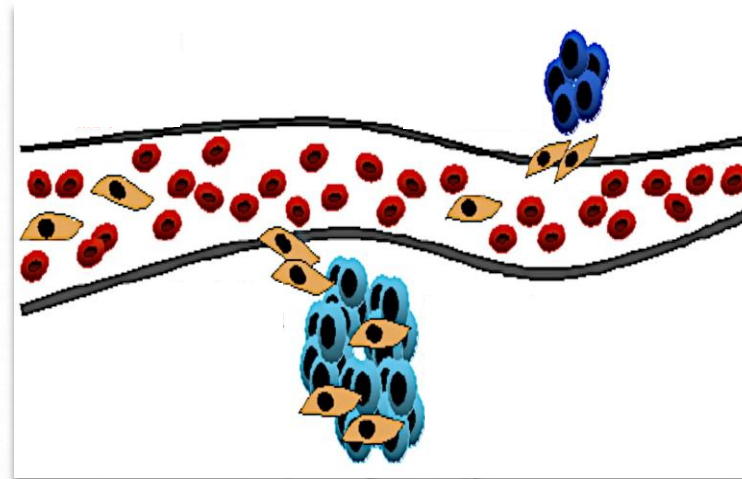
MSCs as Drug Delivery Vehicles: Supportive Feature

- Easy availability from adult bone marrow donors and other sources
- Low immunogenicity
- Selective homing to sites of inflammation and cancer
- Established biodistribution and toxicology profile
- Cryopreservation for long-term storage
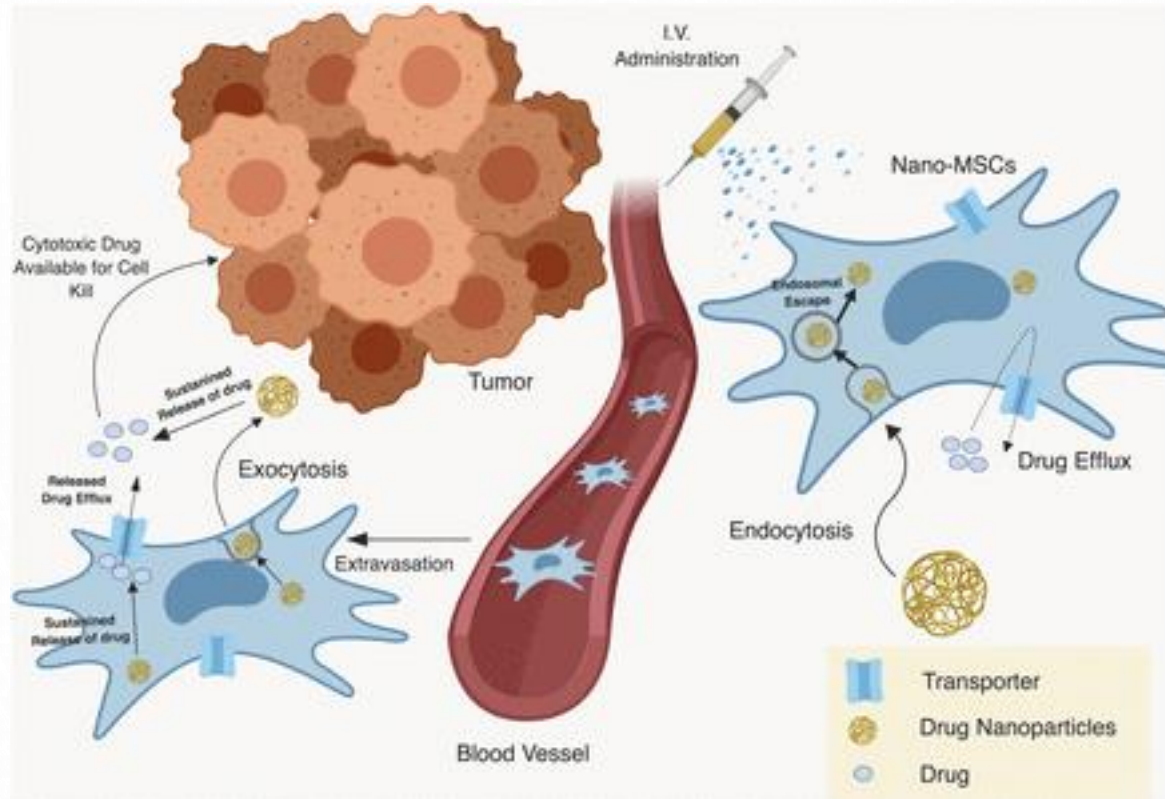
Mechanisms of Tumor Migration
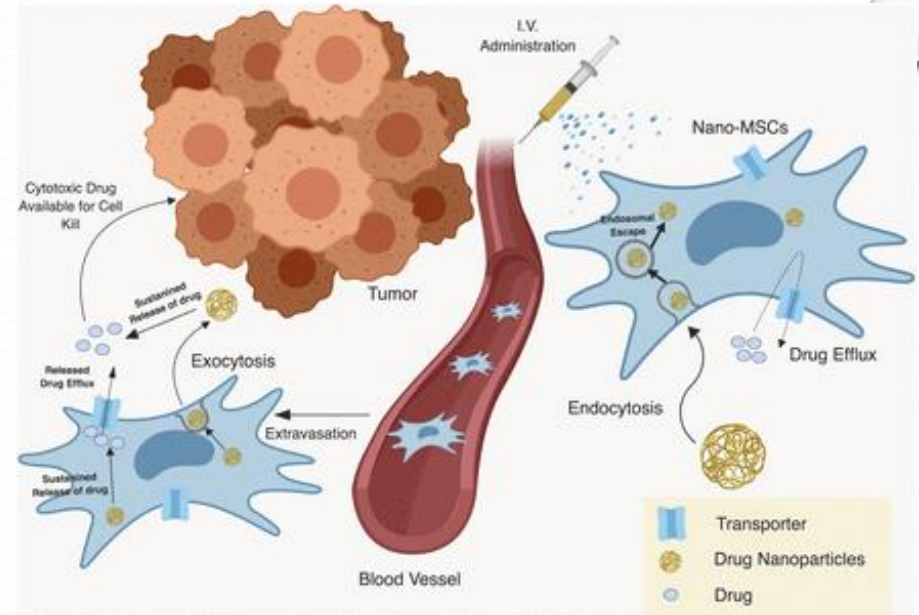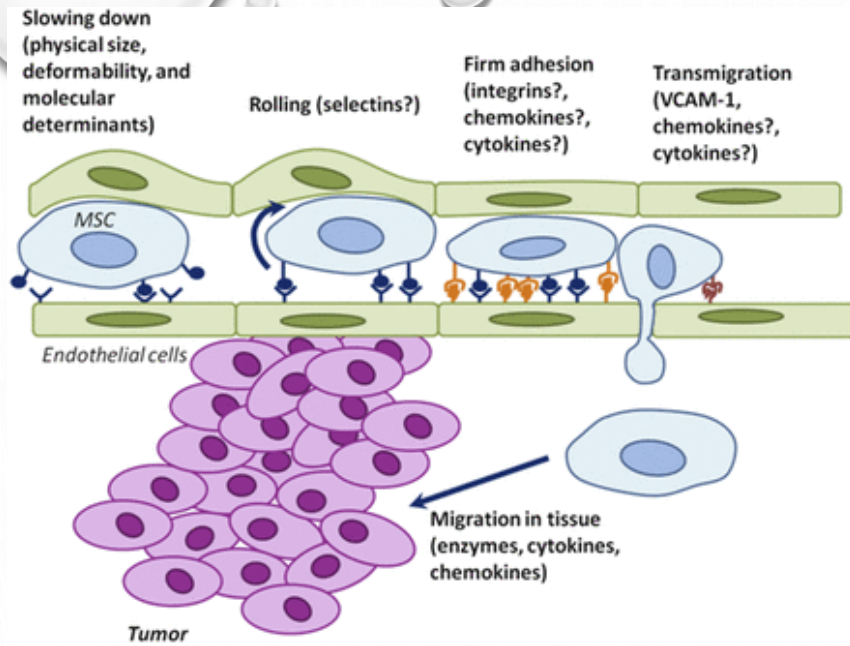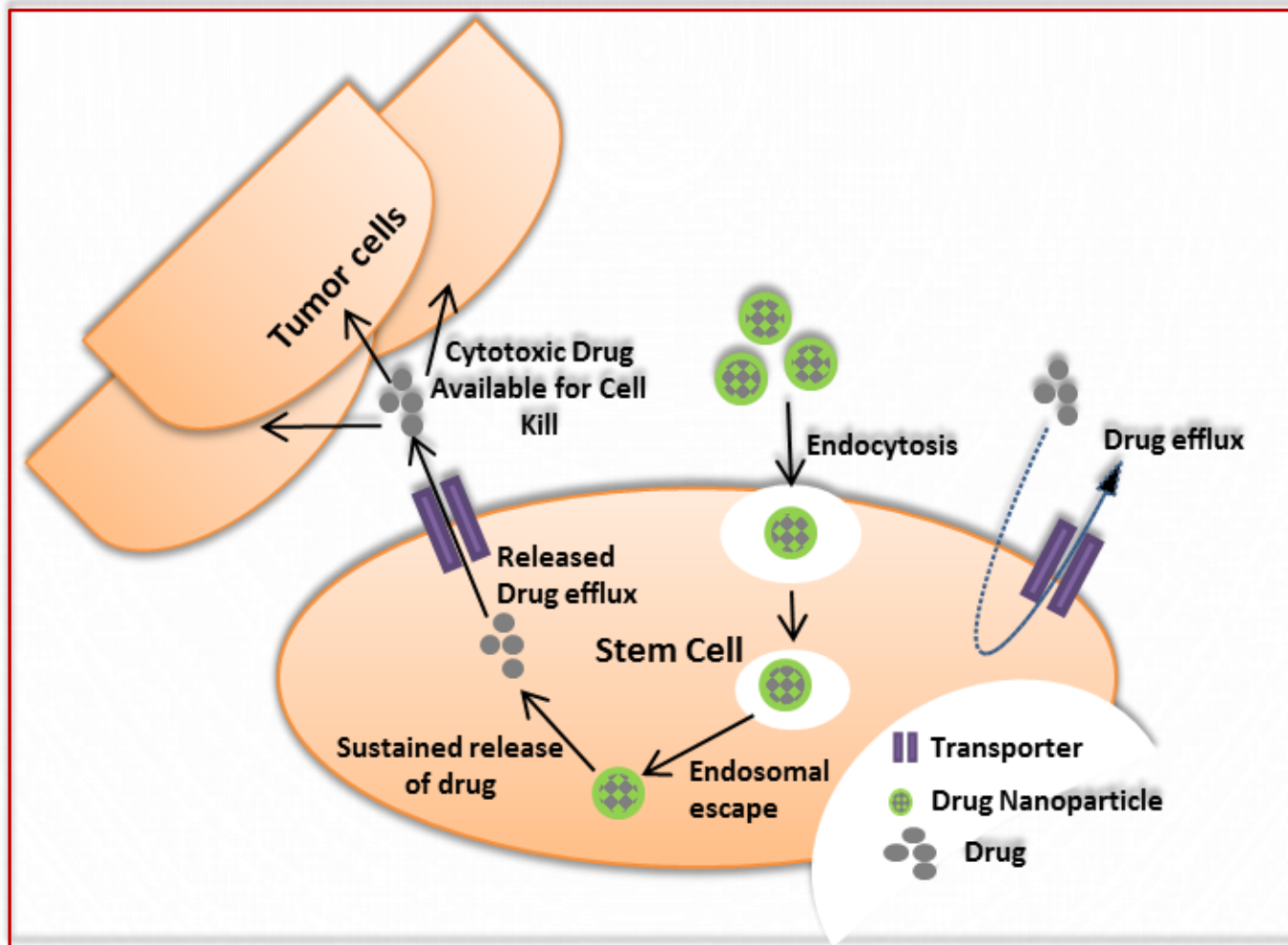

Droujinine et al. Oncotarget 4, 651-664 2013

B. Linju Yen and Men-Luh Yen, J. Cancer Mol. 4: 5-9, 2008

# MSCs as Drug Delivery Vehicles: Limitation

- Drug resistant due to overexpression of efflux transporters
- Poor payload capacity

Nano-engineered MSCs as Drug Carrier: Hypothesis

*Sadhukha et al., J. Control. Rel. 2014, 196, 243-251*

Cell penetrating peptides

❖ Facilitate cellular intake and uptake of molecules

❖ CPPs are typically 5-30 amino acids long

❖ Transactivator of transcription (TAT) peptide has been widely investigated

❖ TAT-derived from the human immunodeficiency virus 1 protein containing 86–102 amino acid residues.

❖ TAT peptide (47 to 57 YGRKKRRQRRR) has been successfully used to deliver biologically active molecules.
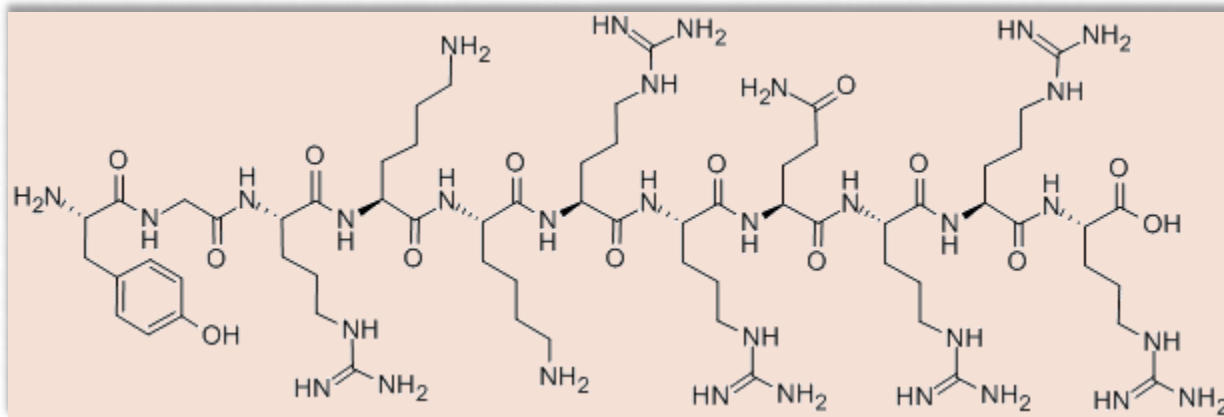
Hypothesis of the study

❖ Surface functionalization of polymeric nanoparticles with TAT peptide will enable

➤ *their improved internalization into and retention by MSCs*

➤ *resulting in enhanced payload carrying capacity*

❖ Covalently conjugated TAT peptide to the surface of nanoparticles encapsulating paclitaxel (PTX) and used these nanoparticles to incorporate paclitaxel (a potent anti-cancer agent) in MSCs.
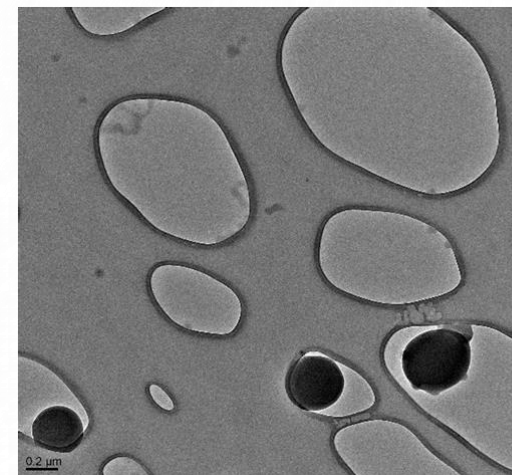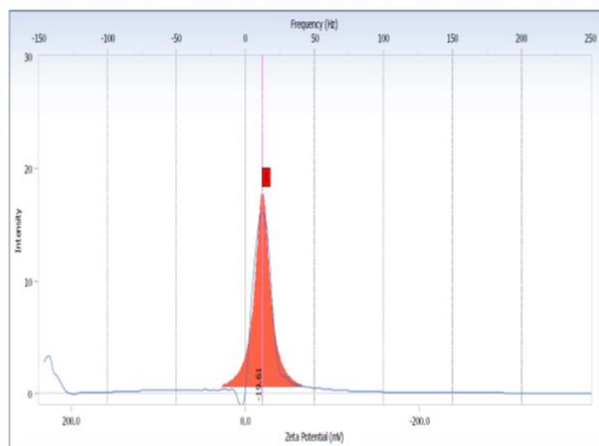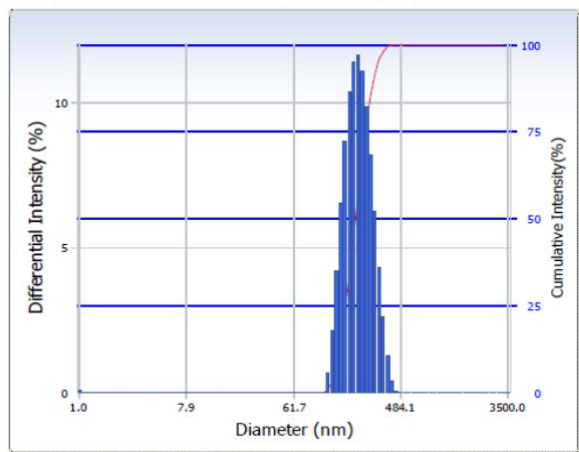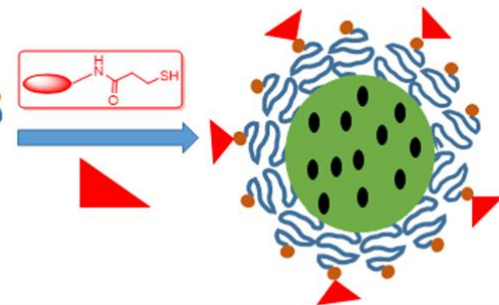
Preparation of TAT-PTX-NPs
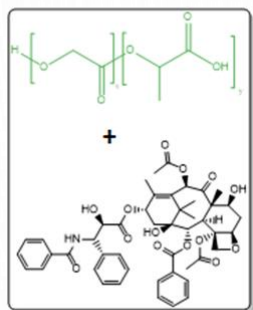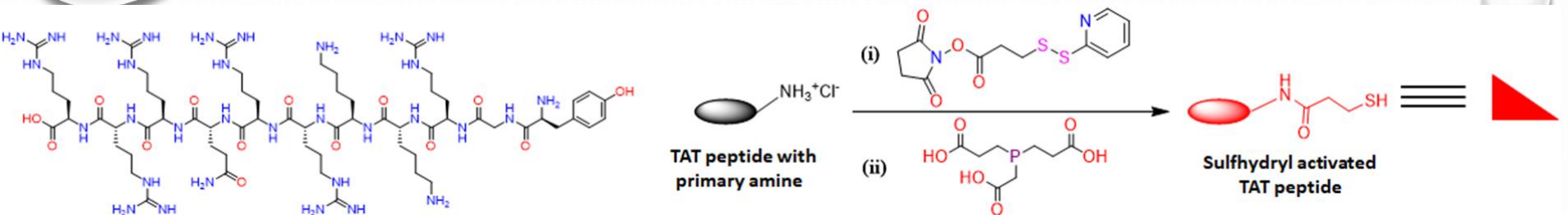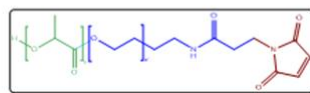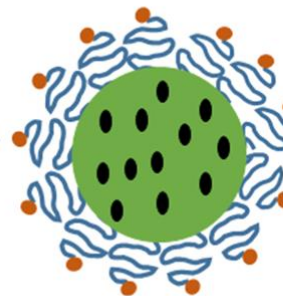
Nano-engineering of MSCs

- PLGA nanoparticles prepared by emulsion-solvent evaporation technique

- Diameter: 225 ± 4 nm, Zeta potential: -15 ± 1.3 mV

- Paclitaxel (PTX) loading: 15-16 % (w/w)

- TAT peptide conjugation to NPs: 57 ± 4% (2.42 ± 0.14 µg/mg of NP).

PTX-loaded NP

37° C, 4 h

Human MSCs

Nano-MSCs
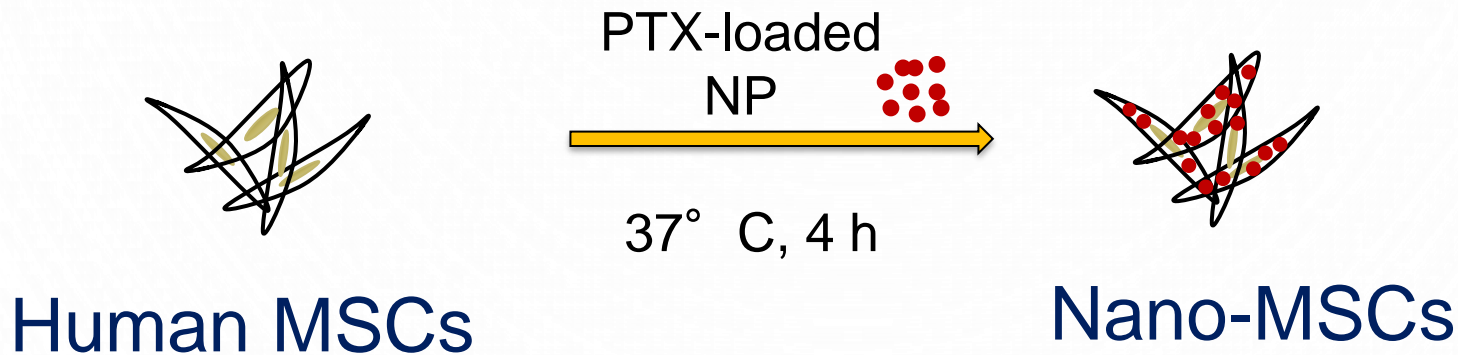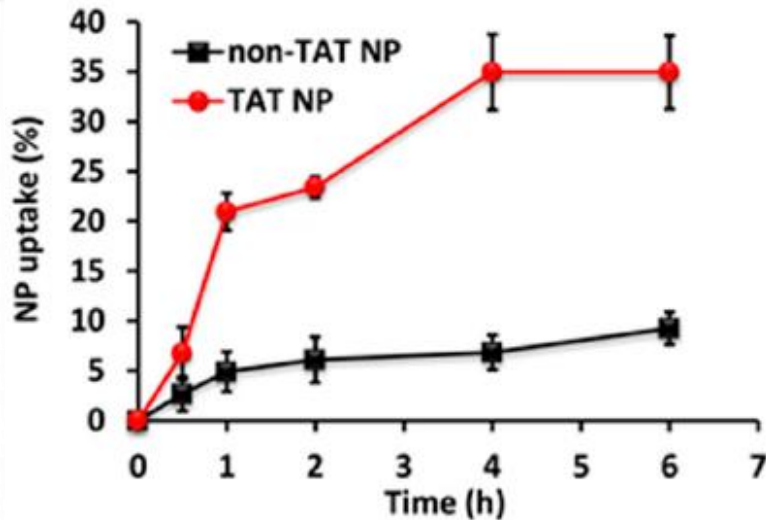
In vitro endocytosis In vitro exocytosis
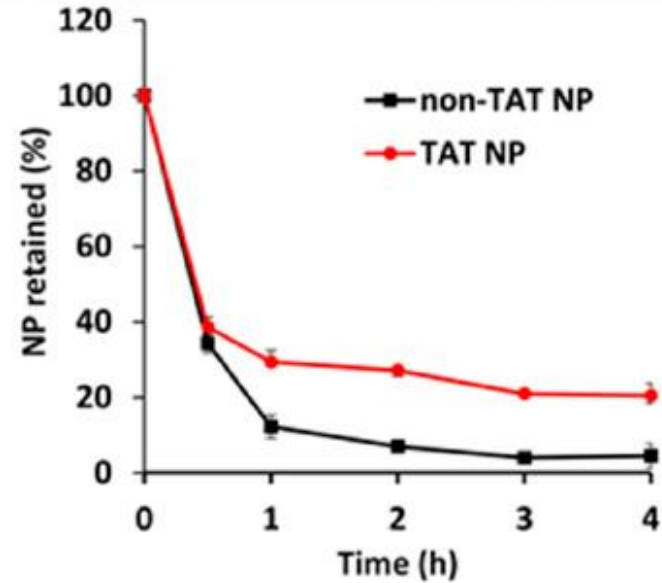
Cytotoxicity profiles of the nano-engineered MSCs, TAT PTX NP and PTX solution in A549 cells

Differentiation (adipogenic and osteogenic) and migration potentials of nano-engineered MSCs

Efficacy of nano-engineered MSCs in inhibiting orthotopic tumor growth

Survival data

Immunohistological analysis

Effect of different treatments on liver function test

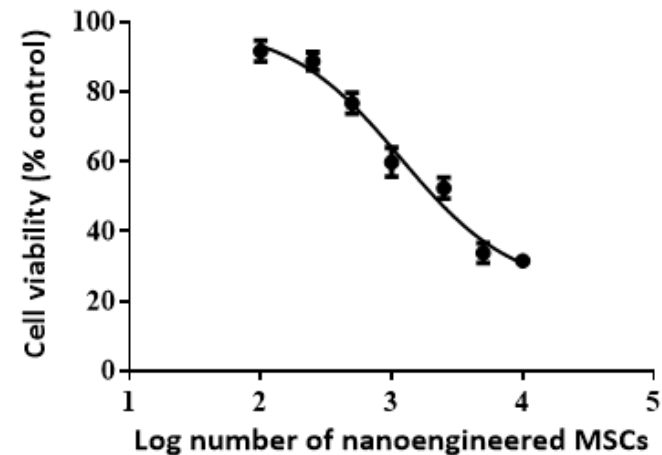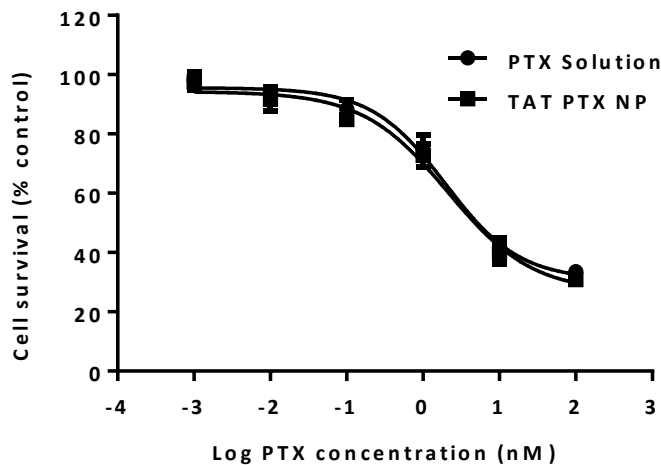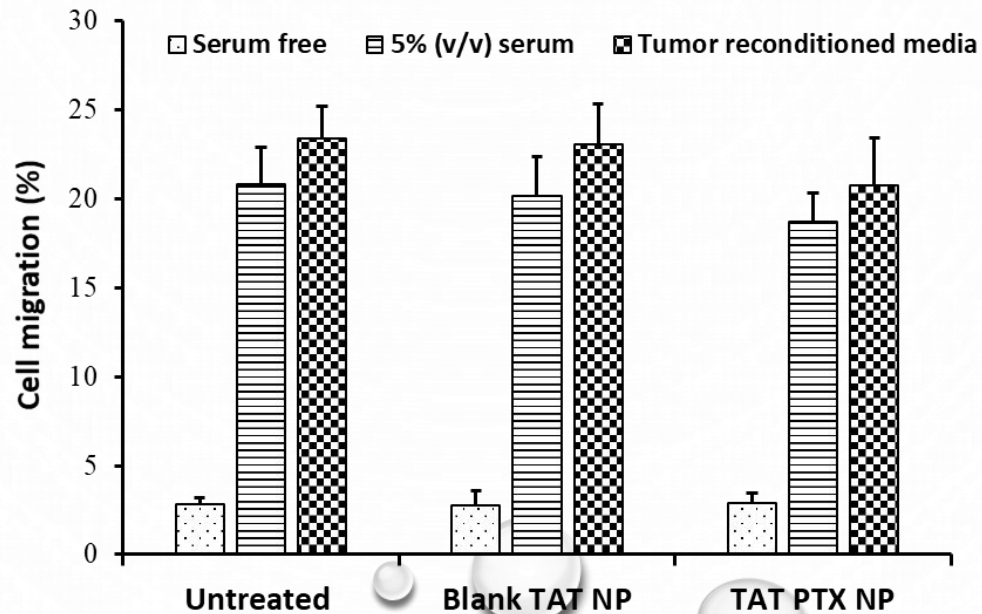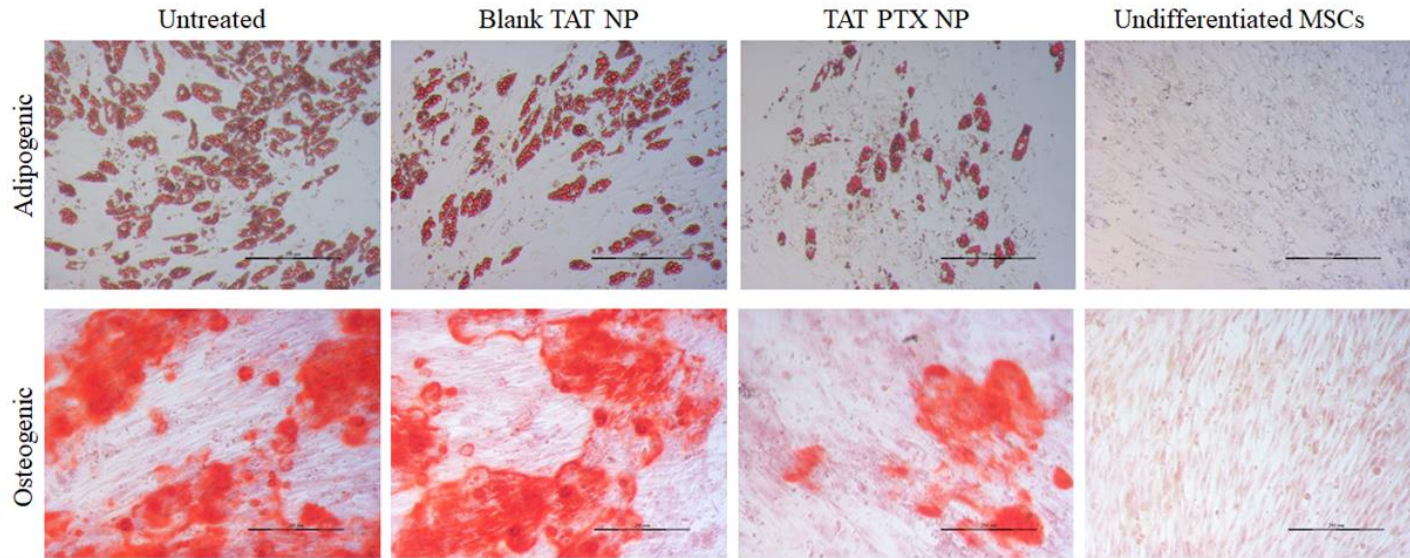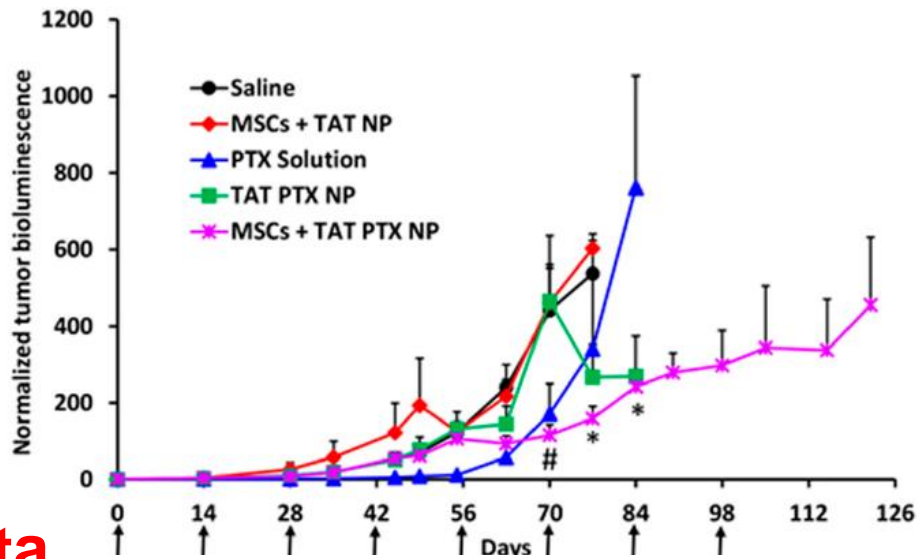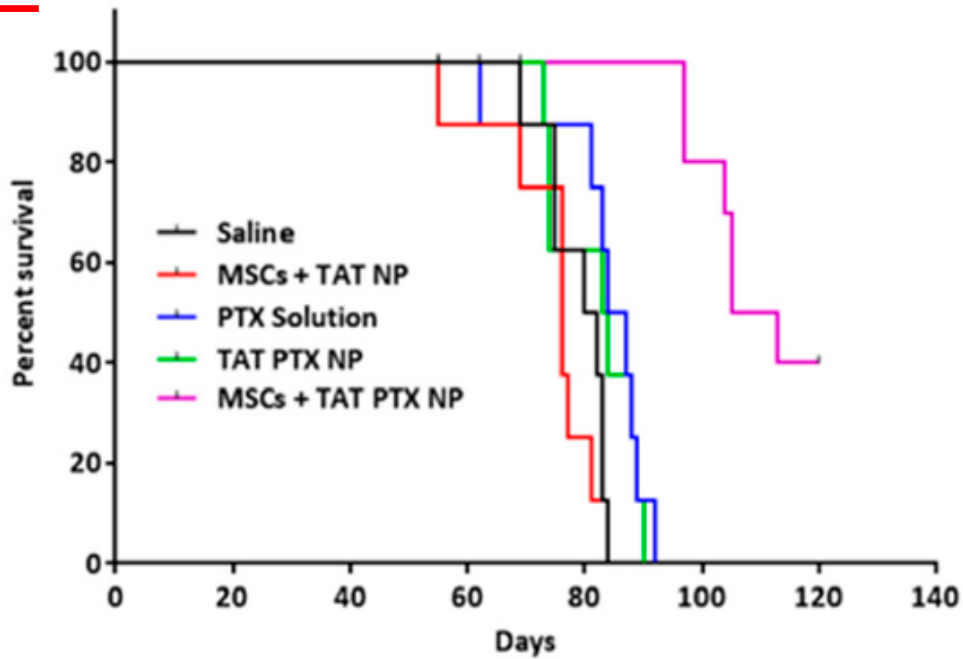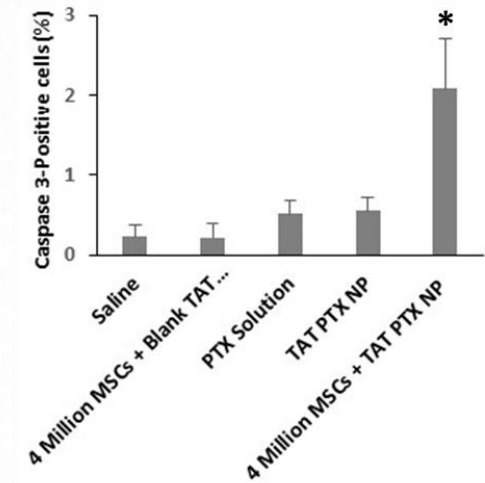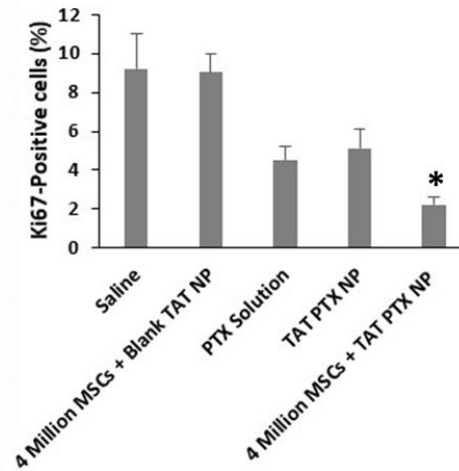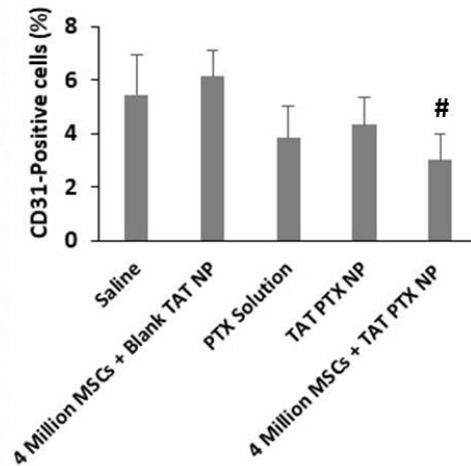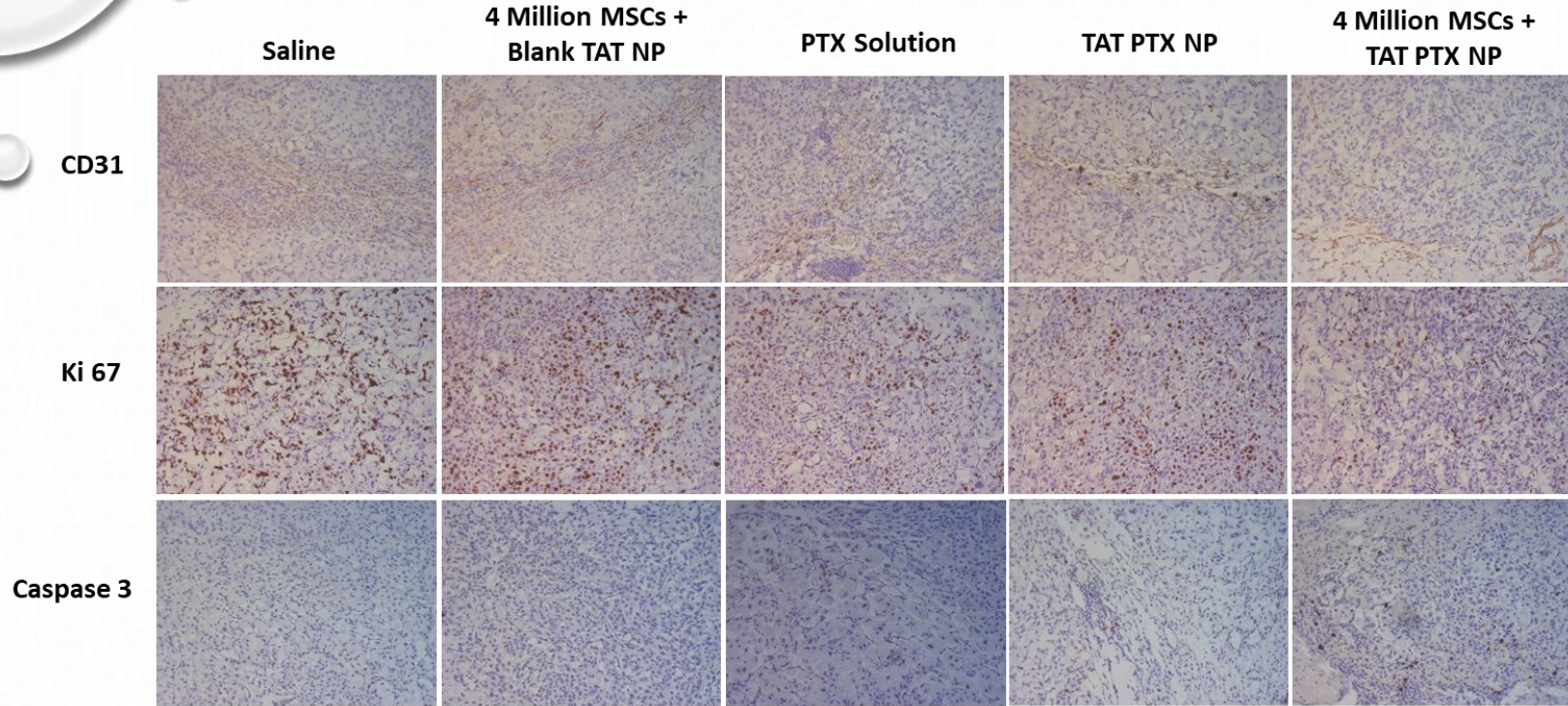| Parameters	Saline		MSCs + TAT NP		TAT PTX NP		MSCs + TAT PTX NP	
	Day 7	Day 18	Day 7	Day 18	Day 7	Day 18	Day 7	Day 18
ALT (U/L)	33.0 ± 7.0	37.0 ± 5.3	33.5 ± 4.7	36.0 ± 5.4	32.5 ± 4.5	33.5 ± 5.5	35.8 ± 6.4	40.0 ± 4.4
AST (U/L)	80.7 ± 9.7	87.3 ± 13.1	85.8 ± 11.5	90.0 ± 10.8	85.7 ± 7.7	90.3 ± 10.0	84.8 ± 11.7	91.5 ± 5.8
GGT(U/L)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
ALP (U/L)	94.3 ± 5.9	100.7 ± 7.1	90.3 ± 4.9	96.8 ± 11.0	95.5 ± 4.2	98.0 ± 16.5	95.0 ± 6.5	100.8 ± 10.3
TP (g/dL)	5.8 ± 0.2	5.4 ± 0.3	5.8 ± 0.3	5.9 ± 0.3	5.7 ± 0.2	5.5 ± 0.1	5.8 ± 0.2	5.3 ± 0.1
ALB (g/dL)	3.5 ± 0.1	3.2 ± 0.1	3.5 ± 0.2	3.4 ± 0.2	3.5 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.4 ± 0.1
GLOB (g/dL)	2.3 ± 0.1	1.9 ± 0.1	2.3 ± 0.2	2.5 ± 0.2	2.1 ± 0.2	2.0 ± 0.1	2.3 ± 0.1	2.4 ± 0.0
A/G	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.0
TBIL (mg/dL)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1

Abbreviations: ALT-alanine aminotransferase; AST-aspartate aminotransferase; GGT-gamma-glutamyl transferase; ALP-alkaline phosphatase; TP-total protein; ALB-albumin; GLOB-globulin; A/G-albumin/globulin ratio; TBIL-total bilirubin.

Effect of different treatments on complete blood count

Parameters	Saline		MSCs + TAT NP		TAT PTX NP		MSCs + TAT PTX NP	
	Day 7	Day 18	Day 7	Day 18	Day 7	Day 18	Day 7	Day 18
WBC ($\times 10^3$ cells/μL)	5.0 ± 1.4	6.3 ± 0.5	4.9 ± 0.8	5.0 ± 0.6	5.3 ± 0.6	4.4 ± 0.2	5.6 ± 1.2	4.7 ± 0.7
RBC ($\times 10^6$ cells/μL)	9.8 ± 0.3	9.7 ± 0.3	9.8 ± 0.2	9.5 ± 0.3	9.8 ± 0.1	9.1 ± 0.3	9.7 ± 0.2	9.1 ± 0.1
HGB (g/dL)	15.4 ± 0.4	15.5 ± 0.6	15.3 ± 0.3	15.0 ± 0.5	15.6 ± 0.2	14.8 ± 0.3	15.1 ± 0.3	14.3 ± 0.3
HCT (%)	50.9 ± 1.1	49.2 ± 1.7	50.2 ± 1.0	46.5 ± 1.6	51.4 ± 0.7	46.1 ± 1.5	49.5 ± 0.9	45.6 ± 0.9
PLT ($\times 10^3$ cells/μL)	840 ± 26	977 ± 50	1106 ± 224	1248 ± 131	914 ± 60	1060 ± 103	1349 ± 238	1234 ± 147

Abbreviations: WBC-white blood cell; RBC-red blood cell; HGB-hemoglobin concentration; HCT-hematocrit; PLT-platelets.

Summary

❖ We demonstrated significantly improved drug loading in MSCs by using TAT functionalized nanoparticles.

❖ These nano-engineered MSCs retained their osteogenic and adipogenic differentiation properties and tumor-tropism.

❖ Nano-engineered MSCs were effective in inhibiting tumor growth and increasing the overall survival in a mouse orthotopic lung tumor model.

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