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## Nanoparticles for Immune Cytokine TRAIL-Based Cancer Therapy

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### Abstract

The immune cytokine tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has received significant attention as a cancer therapeutic due to its ability to selectively trigger cancer cell apoptosis without causing toxicity *in vivo*. While TRAIL has demonstrated significant promise in preclinical studies in mice as a cancer therapeutic, challenges including poor circulation half-life, inefficient delivery to target sites, and TRAIL resistance have hindered clinical translation. Recent advances in drug delivery, materials science, and nanotechnology are now being exploited to develop next-generation nanoparticle platforms to overcome barriers to TRAIL therapeutic delivery. Here, we review the design and implementation of nanoparticles to enhance TRAIL-based cancer therapy. The platforms we discuss are diverse in their approaches to the delivery problem and provide valuable insight into guiding the design of future nanoparticle-based TRAIL cancer therapeutics to potentially enable future translation into the clinic.

### Graphical abstract

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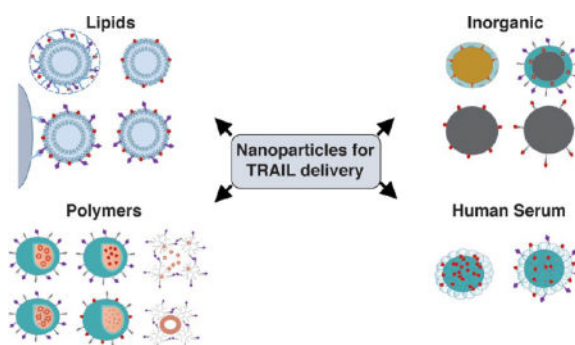
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## Keywords

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The development of therapeutics that induce apoptosis specifically in tumor cells while sparing normal cells has long been a goal for clinicians and researchers to avoid the debilitating effects of conventional chemotherapies. One protein of heightened interest as a cancer-specific therapeutic is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), an innate immune cytokine broadly expressed by immune cells including lymphocytes, natural killer cells, and neutrophils (Figure 1).<sup>1</sup> TRAIL-expressing immune cells play a role in the clearance of diseased cells *in vivo* and have also been shown to suppress tumor initiation and metastasis.<sup>2,3</sup> TRAIL binds to death receptors 4 and 5 (DR4 and DR5) overexpressed on the surface of a range of cancer cells to trigger apoptosis and also binds to three decoy receptors (DcR1, DcR2, DcR3) expressed on cells that do not trigger apoptosis and act as competitive inhibitors (Figure 1).<sup>4,5</sup> TRAIL-based therapeutic approaches have shown high efficacy in numerous preclinical cancer models.

While potent antitumor activity of TRAIL has been observed *in vitro* and in preclinical *in vivo* mouse models, these effects have not been broadly reproduced in clinical trials. Intravenous administration of TRAIL-based therapeutics have been well-tolerated in clinical trials, both as a monotherapy or in combination with conventional chemotherapy and immunotherapy.<sup>6,7</sup> Minimal adverse effects of TRAIL therapeutics have been reported in patients with non-small-cell lung cancer, metastatic pancreatic cancer, basal-like breast cancer, colorectal carcinoma, non-Hodgkin's lymphoma, and multiple myeloma (Table 1),<sup>6,7</sup> indicating that TRAIL-based therapies do not induce the toxic side effects in patients that are commonly faced during chemotherapeutic regimens. While the lack of toxic side effects is promising, studies in cancer patients have demonstrated little efficacy. For example, monomeric, soluble TRAIL protein and agonistic DR4 and DR5 antibodies, used in the first clinical trials, have relatively low potency in terms of inducing apoptosis and thus limit clinical efficacy. Challenges in translating preclinical success into clinical settings include the short circulation half-life of TRAIL (from several minutes in rodents to approximately 60 min in humans), its lack of accumulation in target tumor sites, and the low potency of TRAIL ligands or antibodies in activating DR4 and DR5 on the surface of tumor cells.<sup>7-10</sup> However, the fact that TRAIL has been relatively well-tolerated in clinical trials suggests

there is a therapeutic window for increasing tumor exposure and thus augmenting the efficacy of the ligand.<sup>9</sup>

A key challenge to realizing the broad potential of TRAIL-based therapeutics is the need for safe and effective delivery methods. Recent advances in drug delivery, nanotechnology, and materials science hold promise in overcoming the *in vivo* delivery barriers that hinder TRAIL efficacy in clinical trials. Nanoparticles (NPs) are now utilized to both encapsulate and/or bind TRAIL therapeutics, which can reduce degradation in blood, offer ideal surface presentation of ligands to target cells, and modify the biodistribution and selectivity of drugs to target tissues (Figure 2).<sup>29–32</sup> Gene delivery materials, administered *in vivo* or used to engineer therapeutic cells *ex vivo*, can be exploited for targeted expression and secretion of TRAIL therapeutics at tumor sites *in vivo*.<sup>33,34</sup> Direct modifications of therapeutics with materials such as serum proteins<sup>35</sup> and polymers such as polyethylene glycol (PEG),<sup>36</sup> have shown enhanced circulation half-life, improved tumor targeting, and reduced off-target effects.<sup>37</sup>

Here, we overview recent advances in the design and implantation of NP platforms for TRAIL-based cancer therapeutics. These systems, with reported *in vitro* and *in vivo* efficacy, are diverse in their size, shape, structure, chemistry, and mechanism of action. We highlight the opportunities and challenges in utilizing NPs to advance TRAIL-based cancer therapeutics and provide our analysis of the future outlook for these emerging areas.

## Nanoparticle-Based Approaches

The design and implementation of NPs as drug delivery vehicles for cancer therapy has been extensively studied. NPs encapsulate drugs with limited solubility, protect therapeutics from degradation in blood, offer ideal surface presentation of therapeutic ligands to target cells, and modify the biodistribution and selectivity of drugs to target tissues.<sup>29–32</sup> The first generation of clinically approved nanomedicines including Doxil, a liposomal NP-encapsulated doxorubicin, and Abraxane, an albumin NP-stabilized paclitaxel, reduced toxic side effects and enabled higher tolerated doses of drug in patients, respectively.<sup>38,39</sup> NP-based strategies can exploit either passive or active targeting methods, or a combination of both, to achieve therapeutic delivery to tumors. Through the enhanced permeation and retention (EPR) effect, NPs can passively traverse through large pores in tumor vasculature and accumulate in solid tumors.<sup>40–44</sup> However, the use of passive strategies alone has limitations, as permeability and vessel distribution are not uniform across tumors, preventing the deep penetration and uptake of NPs.<sup>45–47</sup> Active targeting strategies can address this lack of penetration and enhance delivery to tumors by tethering targeting molecules, such as nucleic acids, peptides, and proteins, to the surface of NPs to enable binding to overexpressed receptors or antigens on tumor cells.<sup>40,48–51</sup>

TRAIL is considered to be both a targeting agent and a cell death ligand that triggers apoptosis.<sup>52,53</sup> With negligible toxicity to most normal cells, TRAIL triggers apoptosis by selectively binding to death receptors on the surface of cancerous cells (Figure 1).<sup>52,53</sup> Although the therapeutic potential of TRAIL as a targeting molecule and therapeutic compound are well-established in preclinical studies, challenges remain to enable translation

to the clinic. Recently, the use and development of NP platforms have been explored to improve TRAIL-based therapeutics through (i) enhanced circulation half-life and biodistribution, (ii) overcoming TRAIL resistance, and (iii) improved targeting to diseased tissues.<sup>54</sup> NP-based strategies include (i) the encapsulation of soluble TRAIL protein or TRAIL-encoding plasmids, (ii) TRAIL tethered directly to the surface of NPs to engage TRAIL death receptors on tumor cells *in vivo*, or (iii) combination approaches (Figure 3). Additionally, NP-based approaches utilizing combination therapies of TRAIL with chemotherapeutics or other sensitizers are now being investigated to overcome tumor resistance to TRAIL (Figure 3).<sup>55–57</sup>

## Lipid Nanoparticles

Nanoscale liposomes (Figure 2) have been investigated extensively as drug carriers for cancer therapy, with clinical trials for liposomes resulting in approved therapeutics such as Doxil<sup>58</sup> and DaunoXosome (Kaposi's sarcoma),<sup>59</sup> LipoDox (ovarian and breast cancer and Kaposi's sarcoma),<sup>60,61</sup> Myocet (metastatic breast cancer),<sup>62,63</sup> Marqibo (acute lymphoblastic leukemia),<sup>64</sup> as well as additional drugs currently in clinical trials.<sup>65,66</sup> In addition to their biocompatibility, biodegradability, low toxicity, and low immunogenicity, liposomes can encapsulate both hydrophilic and hydrophobic drugs, protect active compounds from external degradation, and deliver agents into cells. Liposome surface properties, such as membrane fluidity, geometry, hydrophilicity, hydrophobicity, and stability can be optimized to enhance the delivery and presentation of a variety of biomolecules.<sup>67</sup> Modulation of liposome surface properties enables manipulation of blood circulation time, pH sensitivity, entrapment efficiency, and ligand attachment, particularly for those that are most potent in their membrane-bound form.<sup>68</sup> An example modification strategy using PEG, known as PEGylation, has been shown to enhance the serum stability of numerous NP formulations in circulation.<sup>67</sup>

## Enhancing TRAIL Potency via Lipid NP-Based Membrane Display

Given that clinical trials have focused on the administration of TRAIL in soluble form, it has been suggested that the lack of broad efficacy in patients could be due to a lack of potency of the soluble protein, and thus weak engagement of the extrinsic TRAIL apoptotic pathway. Conjugation of TRAIL to the membrane of lipid NPs, in particular, liposomes, has recently been shown by several groups to augment the apoptotic effect of TRAIL in comparison to soluble protein treatment (Figures 2 and 3).<sup>68–73</sup>

In one approach, covalent binding of TRAIL to the liposome membrane using cysteine–maleimide bonds widely increased TRAIL receptor clustering, caspase signaling, and apoptosis across a panel of 479 cell lines.<sup>68</sup> Compared to soluble protein treatment, systemically administered liposomal TRAIL reduced overall tumor volume in a mouse colon cancer xenograft model. These results indicate that membrane display of TRAIL is essential in enabling TRAIL receptor clustering and subsequently inducing apoptosis. Similar effects have also been observed when TRAIL was bound to membranes using a 1,2-dioleoyl-*sn*-glycero-3- $\{[N(5\text{-amino-1-carboxypentyl})\text{-iminodiacetic acid}]\text{succinyl}\}$  (nickel salt) (DOGS-Ni-NTA)-containing liposomal system.<sup>69,71–73</sup> In terms of the apoptotic mechanism,

liposomal TRAIL enhanced caspase 3 activity, an early initiator of apoptosis, and overcame TRAIL resistance in tumor cell lines either overexpressing the antiapoptotic protein Bcl-x<sub>L</sub> or that which lacked expression of the proapoptotic proteins Bax and Bak. Building upon the mechanism behind this response, it was later shown that liposomes increased receptor clustering of DR5, enhanced recruitment of the death-inducing signaling complex (DISC), and triggered caspase activation more efficiently than soluble TRAIL protein.<sup>70</sup> To further enhance targeting for *in vivo* applications, TRAIL-coated liposomes have been combined with membrane-bound targeting agents such as anti-EGFR antibody fragments, which also enhanced DR4 and DR5 activation.<sup>74</sup> Such systems have also been PEGylated to improve pharmacokinetic profiles and *in vivo* delivery.<sup>55</sup>

### Programmable Liposomal Delivery Systems

In addition to exploiting membrane display to enhance apoptosis, liposomes complexed with hyaluronic acid gels have been engineered for programmed delivery and release of both TRAIL and doxorubicin to the tumor microenvironment *in vivo*.<sup>56</sup> A cross-linked hyaluronic acid gel shell was used to encapsulate doxorubicin-containing liposomes coated with both TRAIL and the cell-penetrating peptide R8H3 (Figure 4).<sup>56</sup> Following NP administration, an extracellular enzyme highly expressed in tumors known as hyaluronidase then degraded the core-shell to trigger the release of TRAIL.<sup>56</sup> The elimination of the core-shell exposed R8H3 conjugated to a stearyl chain to anchor the peptide into the liposome membrane *via* hydrophobic and electrostatic interactions, which enhanced cellular uptake of doxorubicin to further trigger apoptosis.<sup>56</sup> In addition to enhanced tumor apoptosis activity *in vitro* and *in vivo*, this NP platform also resulted in increased accumulation of doxorubicin in tumor tissue and enhanced drug circulation half-life compared to free doxorubicin alone or R8H3-modified liposomes formulated with doxorubicin.<sup>56</sup> Strong antitumor activity and high accumulation in the tumor tissues were demonstrated using this formulation, suggesting that these liposome-gel systems can be utilized for the programmed release of TRAIL at target tumor sites that overexpress certain enzymes.<sup>56</sup>

### Combination TRAIL Therapies

The ability of liposomes to encapsulate drugs, in addition to conjugation of TRAIL to the liposome membrane, is advantageous for use in combination therapies to either exert synergistic therapeutic effects or overcome TRAIL resistance (Figure 3B). In one approach, a lipid-based NP system was used to exploit both TRAIL-mediated apoptosis as well as intracellular-triggered apoptosis using the small molecule DM-PIT. The particle system consisted of micelles comprised of both PEG derivatives and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine,<sup>75</sup> a widely used fusogenic lipid that facilitates endosomal escape of NPs.<sup>76</sup> TRAIL was tethered to the surface of micelles, which were loaded with DM-PIT.<sup>75</sup> A combination of TRAIL and DM-PIT enhanced TRAIL-resistant U87MG glioblastoma cell apoptosis compared to micelles loaded with DM-PIT.

Although not as widely explored as polymer-based delivery systems, lipid-based NPs have also been utilized for gene delivery, as TRAIL-encoding plasmid DNA has been combined with the antitumor drug paclitaxel to prepare cationic liposomes functionalized with targeting ligands.<sup>77,78</sup> Collectively, lipid-based NPs have been shown in several studies to

enhance TRAIL potency *via* membrane display, making them an ideal material for improving the delivery of recombinant TRAIL proteins. Such an approach has high translational potential, given that TRAIL is nontoxic and the lipids used in these formulations are already used in liposomal formulations approved by the U.S. Food and Drug Administration (FDA).

## Polymeric Nanoparticles

Polymeric NPs such as micelles, polyplexes, and dendrimers are also being utilized for the delivery of TRAIL-based therapeutics (Figure 2),<sup>40,79</sup> as they are particularly advantageous for the release of therapeutics in a controlled manner, stimuli-responsive drug delivery, and gene delivery.<sup>40,48,80,81</sup> Polymeric NPs can be formulated from a large variety of polymers and copolymers including synthetic biodegradable polymers (poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA)), and natural polymers (alginate and chitosan), enabling them to encapsulate both hydrophilic and hydrophobic drugs. Several polymeric NPs are undergoing clinical trials in the United States,<sup>79</sup> including (i) PLA and PEG NPs containing Paclitaxel to treat breast cancer, lung cancer, and pancreatic cancer (Genexol-PM);<sup>82,83</sup> (ii) PLA and PEG NPs loaded with docetaxel and conjugated to ACUPA (*S,S*-2-[3-[5-amino-1-carboxypentyl]ureido]pentanedioic acid), an inhibitor for the prostate-specific membrane antigen (PSMA) to target non-small-cell lung cancer and prostate cancer;<sup>84</sup> and (iii) a formulation of cyclodextrin polymer, PEG, small interfering RNA (siRNA), and transferrin to target solid tumors (CALAA-01).<sup>85</sup> Drug-loaded polymeric micelles, comprised of self-assemblies of amphiphilic block copolymers consisting of a hydrophobic core as a drug reservoir and a PEG hydrophilic shell, have also demonstrated high translational potential for cancer therapy.<sup>86</sup> Several micelle formulations, including the paclitaxel micelle formulation NK105, are currently under clinical evaluation.<sup>87,88</sup>

## Polymers for Nonviral TRAIL DNA Delivery in Vivo

While significant efforts have focused on the administration of TRAIL in protein form, clinical translation of such approaches is limited by the failure to deliver sufficient protein into the tumor to generate a therapeutic effect.<sup>89-91</sup> As an alternative approach, TRAIL gene therapy can potentially overcome these barriers by delivering TRAIL-encoding plasmid DNA specifically to tumor cells and the surrounding tumor microenvironment, so that they express and secrete TRAIL into tumors locally (Figure 3C).<sup>92,93</sup> However, DNA therapeutics face several barriers to successful *in vivo* delivery. Among those are the degradation of nucleic acids by endonucleases in the bloodstream as well as in tissues.<sup>94,95</sup> Plasmid DNA is also cleared from the bloodstream rapidly, as its half-life has been estimated to be 10 min following intravenous injection in mice.<sup>96</sup> For this reason, the entrapment of DNA in NPs is a particularly attractive approach both to provide protection from endonuclease degradation and to extend the circulation half-life of DNA. NPs can also aid in avoiding renal clearance from the blood due to size-based effects, and preventing nonspecific interactions *via* PEGylation of NPs. Additionally, NPs can also extravasate from the bloodstream to reach target tissues *via* various targeting-based approaches, and mediate cell entry and endosomal escape. Finally, DNA has to be transported to the nucleus to enable access to transcriptional machinery.<sup>94</sup> In the context of TRAIL gene therapy, several

polymer-based approaches including polyethylenimine (PEI) and cationic dendrimers have been utilized for *in vivo* delivery.

### PEI-Based TRAIL Gene Delivery Systems

PEI is a widely used cationic polymer for DNA and RNA delivery due to its high transfection efficiency and its ability to induce endosomal escape (Figure 3C).<sup>80,97</sup> In the context of TRAIL-based cancer therapy, PEI has been complexed with TRAIL-encoding plasmids to enhance delivery to cells, which then secrete the therapeutic locally to induce tumor apoptosis. For example, a peptide-polymer conjugate consisting of an epidermal growth factor receptor (EGFR) targeting peptide (GE11), PEG, and PEI (GE11-PEG-PEI) was developed to form NPs for targeted TRAIL gene delivery.<sup>98</sup> PEI and GE11 were bound using a heterobifunctional PEG (maleimide-PEG-NHS), where the amino group of GE11 was coupled to the NHS group to obtain GE11-PEG-maleimide, which was then coupled with PEI using the maleimide amino reaction to obtain GE11-PEG-PEI.<sup>98</sup> To formulate NPs, GE11-PEG-PEI was further complexed with a TRAIL-encoding plasmid *via* electrostatic interactions.<sup>98</sup> Both *in vitro* and *in vivo*, the modified NPs significantly increased gene transfection efficiency and inhibited Hep-2 cell tumor growth in mice compared to unmodified NPs.<sup>98</sup>

Alternative strategies have utilized PEG-PEI polymer conjugates functionalized with either RGD peptides targeting integrins expressed by gliomas (RGD-PEG-PEI),<sup>99</sup> or small molecules targeting PSMA expressed on prostate cancer cells<sup>100</sup> to form NPs for TRAIL gene delivery. In a mouse intracranial glioblastoma xenograft model, RGD-PEG-PEI NPs administered systemically 11 days post-tumor implantation (four injections over days 11–17), in combination with paclitaxel-loaded micelles targeting nicotine acetylcholine receptors that facilitate blood-brain barrier (BBB) crossing (four injections over days 4–16), enhanced the median survival of mice (33.5 days) compared to treatment with paclitaxel micelles alone (25.5 days).<sup>99</sup> Similarly, PSMA-targeting NPs administered systemically induced TRAIL expression in a mouse prostate cancer xenograft model.<sup>100</sup> In combination with the nontoxic prodrug 5-fluorocytosine (5-FC), which converts to the chemotherapeutic agent 5-fluorouracil (5-FU) in the presence of the prodrug enzyme bacterial cytosine deaminase bound to the NP surface, the combination therapy induced significant inhibition of tumor growth in a mouse prostate cancer xenograft model.<sup>100</sup> The conversion of the drug was also measured using noninvasive functional magnetic resonance spectroscopy, indicating that the NPs can also be utilized as a theranostic platform.<sup>100</sup> Collectively, PEI has been incorporated into several NP platforms to enhance TRAIL gene delivery in multiple mouse models. However, the toxicity of high molecular weight PEI is a major limitation that must be considered in therapeutic regimens where continuous dosing of NPs are required.<sup>97</sup>

### Dendrimer-Based TRAIL Gene Delivery Systems

Dendrimers have been widely used as scaffolds for drug delivery, due to their highly branched, globular architecture with well-defined molecular weight and arms assembled from a multifunctional core.<sup>101</sup> Cationic polyamidoamine (PAMAM) dendrimers have been utilized as TRAIL gene delivery vehicles due to their positive charge, high transfection

efficiency, and pH buffering ability, which facilitates cargo release from endosomes.<sup>81,102–104</sup> As a means to enable TRAIL gene delivery to gliomas, PAMAM dendrimers complexed with TRAIL-encoding plasmids were conjugated to Angiopep-2, a peptide with high transcytosis capacity capable of crossing the BBB, using a heterobifunctional PEG polymer.<sup>102</sup> TRAIL gene therapy using systemically administered, targeted PAMAM NPs extended survival of a mouse intracranial glioma xenograft model compared to treatment with nontargeted NPs or Temozolomide, an FDA-approved chemotherapeutic for the treatment of brain cancer.<sup>102</sup> PAMAM NPs have also been functionalized with various ligands targeting transferrin receptors, which are highly expressed on many tumor cell types, to enhance TRAIL gene delivery and extend survival in multiple tumor-bearing mouse models.<sup>103,104</sup>

Dendrimer-based NPs have also been utilized for the co-delivery of TRAIL-encoding plasmids and chemotherapeutics in tumor-bearing mouse models.<sup>104,105</sup> In one approach, doxorubicin was intercalated with a TRAIL-encoding plasmid followed by condensation using dendrigraft poly-L-lysine polymers to form NPs.<sup>105</sup> NPs were functionalized with a choline derivate, which has high BBB choline transporter activity and is overexpressed on glioma cells, using a heterobifunctional PEG to facilitate targeting.<sup>105</sup> Systemic co-delivery of doxorubicin and the TRAIL-encoding plasmid using NPs extended survival of a mouse intracranial glioma xenograft model, compared to doxorubicin and NP-encapsulated plasmid treatments alone, while also reducing the dosage of doxorubicin required to achieve therapeutic efficacy.<sup>105</sup>

Collectively, these preclinical studies indicate that dendrimer-based NPs are not only effective in the delivery of TRAIL-encoding plasmids and the transfection of target cells *in vivo*, but can also be complexed with chemotherapeutics for combination therapies. While considered less toxic than PEI, dendrimers such as PAMAMs are not biodegradable and exhibit cytotoxicity due to their high density of positive charge.<sup>106</sup> Looking forward, biodegradable structures such as dendrigraft poly-L-lysine, should they achieve high transfection efficiency, could be investigated further as delivery vehicles with reduced *in vivo* toxicity to enable continuous dosing. Beyond dendrimers, NP systems consisting of both cationic polypeptides and lipic acid have been utilized in the co-delivery of doxorubicin and TRAIL-encoding plasmids.<sup>107</sup> The polypeptides effectively condensed plasmid DNA, while lipic acid enabled self-assembly into a micelle-like nanostructure.<sup>107</sup> Given that the NP system exerted potent tumor cell apoptosis with minimal systemic toxicity, such systems could also be explored as an alternative to PEI and PAMAM-based gene delivery systems.

### **Polymeric NPs as Mechano-amplifiers of TRAIL-Mediated Apoptosis**

Beyond gene delivery, polymeric NPs have also been utilized to sensitize tumor cells to TRAIL-mediated apoptosis in the presence of physiological forces.<sup>108</sup> Recent work from our lab has indicated that, in addition to chemical sensitizers, tumor cells become sensitized to TRAIL-mediated apoptosis in the presence of mechanical forces.<sup>109</sup> Mechanical force in the form of fluid shear stress, mimicking shear forces of those in the microvasculature *in vivo*, enhanced cancer cell TRAIL-mediated apoptosis *in vitro* compared to similar treatments



under static conditions.<sup>109,110</sup> Inspired by this observation, we developed polymeric NP mechanical amplifiers that exploit *in vitro* and *in vivo* physical forces to enhance TRAIL-mediated apoptosis.<sup>108</sup> Mechanical amplifiers, consisting of biodegradable, FDA-approved PLGA particles tethered to the tumor cell surface *via* PEG linkers, increased the apoptotic effect of TRAIL on tumor cells under fluid shear exposure by as much as 50% compared with treatment under static conditions.<sup>108</sup> While further *in vivo* studies will be required to assess if this phenomenon can be exploited therapeutically, initial work in mice has shown that pretreatment of tumor cells with systemically administered, targeted NPs followed by treatment with TRAIL reduced circulating tumor cells in blood and overall tumor cell burden by over 90%.<sup>108</sup> Furthermore, the approach reduced solid tumor growth of a mouse prostate cancer xenograft model when treated in combination with the antioxidant resveratrol, potentially opening applications for nanotechnology to enhance TRAIL-mediated signaling and apoptosis in the presence of physical forces *in vivo*.<sup>108</sup>

## Iron Oxide Nanoparticles

Iron oxide NPs are a class of biocompatible inorganic NPs (Figure 2) with ideal magnetic properties that have been traditionally utilized as contrast agents for magnetic resonance imaging (MRI).<sup>111,112</sup> However, recent advances have now opened the door for cellular-specific targeting, drug delivery, and multimodal imaging using iron oxide NPs.<sup>113</sup> Iron oxide NPs can induce tumor cell death through hyperthermia through the generation of heat by alternating magnetic fields, improve antitumor drug or gene delivery in the presence of an extracorporeal magnetic field, and serve as MRI contrast agents.<sup>114,115</sup> FDA-approved iron oxide NPs currently used in the clinic include iron oxide NPs coated with a carbohydrate shell (Ferumoxytol or Feraheme) to treat iron deficiency anemia.<sup>116</sup>

### Iron Oxide NP-Mediated TRAIL Gene Delivery

In the context of TRAIL therapy, iron oxide NPs have been utilized in combination with polymeric materials for *in vivo* TRAIL gene delivery to tumors. In a recent study, iron oxide NPs were coated with a chitosan-PEG-grafted PEI copolymer, covalently bound to chlorotoxin, and complexed with a TRAIL-encoding plasmid.<sup>112</sup> Chlorotoxin, which binds to matrix metalloproteinase-2 and Annexin A2 on glioblastoma cells, was tethered to the surface of NPs *via* thiol-specific conjugation.<sup>112</sup> Modified iron oxide NPs delivered TRAIL-encoding plasmid DNA to induce apoptosis in glioblastoma cells and significantly reduced tumor growth in a mouse xenograft model of glioblastoma compared to treatment with control plasmid DNA.<sup>112</sup> In attempt to exploit their magnetic properties, another study showed that similar PEI-modified NP gene delivery systems, under the presence of a magnetic field, enhanced NP uptake and guided NP transport to tumors in mice. The approach was utilized as a means to induce TRAIL expression and secretion within the local microenvironment to reduce overall tumor burden.<sup>117</sup>

### Magnetic NP Switches for TRAIL-Mediated Apoptosis

Beyond gene delivery, iron oxide NPs have also been utilized to create a “magnetic switch” for tumor cell apoptosis.<sup>118</sup> Rather than conjugating TRAIL protein to the NP surface, TRAIL receptor DR4 targeting antibodies were conjugated to the surface of iron oxide NPs

to enable binding to tumor cell TRAIL receptors.<sup>118</sup> Upon application of a magnetic field, iron oxide NPs bound to the surface caused TRAIL DR4 receptors to cluster together *in vitro* and in zebrafish,<sup>118</sup> which triggered tumor cell apoptosis in a similar fashion to the TRAIL protein itself. Such an approach can be of use when it is necessary to turn on or turn off an apoptotic signaling *in vitro* or *in vivo*. In addition to TRAIL receptor antibodies, TRAIL proteins have also been conjugated to the surface of iron oxide NPs,<sup>89</sup> which extended the survival of mouse glioma xenograft model compared to treatment with TRAIL protein alone.

Taken together, the magnetic properties of iron oxide can be exploited in TRAIL-based therapies by boosting gene delivery in the presence of a magnetic field, while also promoting the clustering of tumor cell TRAIL receptors as a means to induce apoptosis in the absence of TRAIL protein. Furthermore, their favorable biocompatibility, biodegradability, and use as image contrast agents could open up future applications for TRAIL-based NPs as theranostics.<sup>40,48,119</sup>

## Gold Nanoparticles

Gold NPs are a class of inorganic NPs (Figure 2) that offer an ideal combination of physical, chemical, optical, and electronic properties that have proven useful for drug delivery, as well as for use as imaging agents to diagnose disease and guide surgical procedures.<sup>120</sup> Of note, the optical and electronic properties of gold NPs have enabled, in addition to therapeutic delivery, simultaneous photothermal therapy where the irradiation of NPs with near-infrared light at specific frequencies leads to rapid tumor cell death with minimal damage to surrounding tissue.<sup>119–122</sup> A notable clinical advantage of gold NPs is their classification by the U.S. FDA as a medical device for photothermal therapy applications, enabling accelerated approval processes with reduced cost.<sup>120</sup> Beyond photothermal therapy, several gold NP platforms are currently undergoing clinical trials for drug delivery applications, including PEGylated gold NPs (Aurimmune) surface functionalized with tumor necrosis factor alpha (TNF $\alpha$ ) to induce tumor cell death.<sup>123</sup> Most recently, a spherical nucleic acid platform consisting of short strands of RNA densely arranged on the surface of spherical gold NPs were approved by the U.S. FDA as an investigational drug, in an early stage clinical trial to treat glioblastoma multiforme.<sup>124</sup> These “spherical nucleic acids” were the first to demonstrate nucleic acid delivery by intravenous injection to silence critical oncogenes in mouse models of intracerebral glioblastoma, reducing tumor progression and extending survival significantly.<sup>125</sup>

While less studied compared to lipid and polymer-based systems, gold NPs have recently been explored for use as sensitizers to TRAIL-mediated apoptosis as well as for the delivery of TRAIL-encoding plasmids.<sup>126,127</sup> A recent study discovered that gold NPs combined with soluble TRAIL protein treatment significantly increased tumor cell apoptosis compared to soluble TRAIL treatment alone, mediated *via* dynamin related protein 1-based mechanisms.<sup>126</sup> In a non-small-cell lung cancer xenograft mouse model, systemic administration of both gold NPs and soluble TRAIL significantly reduced overall tumor burden compared to treatment with TRAIL or gold NPs alone, indicating that solid tumors can be sensitized to TRAIL-mediated apoptosis *via* simultaneous administration of gold

NPs.<sup>126</sup> In terms of gene delivery, gold NPs were recently functionalized with TRAIL-encoding plasmids in combination with the nucleustargeting steroid dexamethasone (Figure 5).<sup>127</sup> PEI-coated gold NPs were functionalized with plasmid DNA *via* electrostatic interactions, after which NPs were functionalized with a PEI-dexamethasone conjugate *via* similar interactions with surface bound DNA to form a sandwich-like NP structure (Figure 5).<sup>127</sup> The platform significantly enhanced TRAIL gene delivery to tumor cells and reduced overall tumor burden in a mouse liver cancer xenograft model, compared to nontargeted formulations and PEI-DNA complexes alone.<sup>127</sup> Collectively, the use of gold NPs as sensitizers to TRAIL-mediated apoptosis and for use in DNA delivery potentially hold promise, but overall remains an understudied area that requires further investigation. Gold NPs are also nondegradable, and their potential for toxicity upon continuous dosing will require further study. Despite potential limitations, their use in photothermal therapy and as imaging agents could enable future TRAIL combination therapies as well as theranostic-based approaches.

## Human Serum Albumin (HSA) Nanoparticles

HSA is a small, abundant protein in blood with desirable properties for NP formulations including biodegradability, biocompatibility and nonimmunogenicity.<sup>35,128,129</sup> In addition to reducing systemic toxicity and enhancing the tolerance of high antitumor drug doses (Figure 2), HSA-based NPs have been shown to (i) enhance permeability and retention of macromolecules in tumors, and (ii) induce high intracellular penetration through binding of tumor receptor gp60 and the glycoprotein SPARC (secreted protein acidic and rich in cysteine) in the tumor interstitial space.<sup>129</sup> Several HSA products are in clinical trials or are approved for clinical use in diagnostics and treatment of cancer and rheumatoid arthritis, including Abraxane, Alunex, and <sup>99m</sup>Tc-aggregated albumin (Nanocoll and Albures).  
130–132

In the context of TRAIL delivery, HSA has been utilized to create negatively charged NPs complexed with TRAIL using a macromolecular cross-linking desolvation process. *In vivo*, HSA NPs induced a 9-fold increase in TRAIL circulation half-life in a mouse melanoma xenograft model.<sup>133</sup> Other approaches using HSA NPs have been utilized to overcome multidrug resistance. One such mechanism for drug resistance is the overexpression of efficient transporter proteins on the surface of tumor cells, which can release therapeutics such as doxorubicin from cells, as a means of reducing the therapeutic effect.<sup>57</sup> To overcome drug resistance, TRAIL and the targeting ligand transferrin were conjugated to primary amine-functionalized HSA NPs while simultaneously loaded with doxorubicin.<sup>57</sup> Treatment with targeted HSA NPs enhanced apoptosis in a range of multidrug resistant tumor cell lines, and localized within mouse tumor xenograft models.<sup>57</sup> While the exact mechanism remains unclear, the synergistic cytotoxic effects of TRAIL and doxorubicin could be due to reduced cellular FLICE-like inhibitory protein (c-FLIP) expression and the upregulation of TRAIL receptors DR4 and DR5.<sup>57</sup> In summary, recent work suggests that HSA NPs are particularly advantageous as potential long-acting TRAIL therapeutic agents with extended circulation half-life. However, compared to membrane-bound TRAIL liposomal systems, HSA NPs with covalently bound TRAIL will likely be less potent, due to their inability to induce TRAIL receptor clustering.

## Interfacing Nanoparticles with Circulating Cells for Advanced TRAIL Delivery Systems

### Leveraging Immune Cells for NP-Based TRAIL Delivery

In an effort to enhance the circulation half-life and targeting of NPs, circulating blood cells have recently been employed in a variety of therapeutic applications to enable the “hitchhiking” of NPs.<sup>134–136</sup> The concept of cellular hitchhiking is centered on binding NPs to the cell surface, and leveraging the innate ability of red blood cells and immune cells to (i) transport throughout the circulation while avoiding clearance and uptake by the immune system, (ii) remain relatively inert in the circulation until their function is required, and (iii) perform specific functions, including nutrient delivery to tissues, clearance of pathogens, and immune system surveillance.<sup>134</sup>

In the context of TRAIL delivery, liposomes functionalized with TRAIL and the vascular adhesion receptor E-selectin<sup>137,138</sup> *via* noncovalent nickel coordination have been used to bind to the surface of circulating immune cells *in vivo* (Figure 6).<sup>139,140</sup> These NP-functionalized immune cells are utilized to target and kill circulating tumor cells (CTCs) in the bloodstream, as a means to reduce metastatic tumor formation.<sup>139</sup> *In vivo*, the delivery system coated circulating immune cells in blood with negligible toxic effects.<sup>139</sup> The approach killed most CTCs within 2 h of entering the bloodstream and was more effective than targeting CTCs with liposomes functionalized with TRAIL alone.<sup>139</sup> Beyond these benefits, TRAIL displayed a significantly longer circulation half-life due to its tethering to the surface of circulating immune cells.<sup>140</sup> Given that CTCs in the bloodstream are rare and difficult to target,<sup>141–143</sup> interfacing NP therapeutics with immune cells is a potentially promising approach, as they display similar migration characteristics as CTCs and thus can increase the opportunities for TRAIL to bind and induce apoptosis (Figure 6). Furthermore, the ability to functionalize immune cells *in vivo* avoids the significant time, cost, and labor burden of conventional *ex vivo* cellular engineering approaches, which are commonly utilized in most cellular hitchhiking approaches.<sup>134</sup>

### Platelet Membrane-Coated NPs for TRAIL Delivery

In addition to whole cells, cellular membranes are now being interfaced with NPs to improve targeting, increase circulation time, and decrease immunogenicity, while retaining and exploiting the biological functions inherent to cells.<sup>119,144–147</sup> One recent development is the “cloaking” of NPs with platelet membranes, by fusing human platelet membranes freshly isolated from blood with biodegradable PLGA NPs.<sup>144</sup> Platelet membrane-cloaked NPs exhibit several benefits compared to uncoated NPs, including reduced cellular uptake by macrophages and a lack of NP-induced complement activation, while also displaying platelet-mimicking properties including selective adhesion to damaged endothelium as well as enhanced binding to platelet-adhering pathogens.<sup>144</sup>

Platelet membrane-coated NPs have recently been employed for *in vivo* TRAIL delivery.<sup>148,149</sup> In a similar approach, doxorubicin-loaded nanogels were cloaked with platelet membranes overexpressing P-selectin, which bind to CD44 receptors upregulated on cancer cells, followed by TRAIL conjugation to the surface (Figure 7).<sup>148</sup> Platelet-cloaked NPs

exhibited enhanced adhesion and uptake in cancer cells and significantly reduced tumor burden in a mouse breast cancer xenograft model compared to noncoated TRAIL doxorubicin NPs.<sup>148</sup> In another study inspired by receptor–ligand interactions between activated platelets and cancer cells in the circulation, biocompatible silica particles were functionalized with platelet membranes and conjugated with TRAIL to target CTCs in blood.<sup>149</sup> The silica–platelet membrane approach significantly decreased lung metastases in a mouse breast cancer xenograft model compared to uncoated silica NPs (Figure 8).<sup>149</sup> While this approach has demonstrated significant promise in preclinical studies, it must be taken into consideration that tumor cells are remarkably heterogeneous, and may not express adhesion molecules for activated platelet receptors. Thus, further investigation is required to determine the specific types of metastatic cancer that such a therapeutic approach could target.<sup>149</sup> Additionally, the time, cost, and labor burden of isolating platelets, along the reproducibility of such approaches, will need to be further investigated.

## **Ex Vivo Nanoparticle-Based Genetic Engineering of Cells for TRAIL Therapy**

### **Mesenchymal Stem Cell-Based Therapies**

Beyond *in vivo* delivery of TRAIL-encoding plasmids, which can have significant off-target effects when administered systemically in NP form, cells can also be genetically engineered *ex vivo* to express and secrete TRAIL (Figure 9). These cells can then be readministered *in vivo*, where their ability to migrate to diseased tissues is utilized to secrete TRAIL locally (Figure 9). One major cell type used for such approaches are mesenchymal stem cells (MSCs), a form of nonhematopoietic, multipotent stem-like cells currently under investigation as cell-based therapeutics for a range of diseases. In terms of TRAIL delivery, MSCs can be engineered to either secrete or express TRAIL directly on the MSC surface. In contrast to administering TRAIL gene delivery vehicles *in vivo*, engineered MSCs have two distinct advantages: (i) access to isolated, poorly vascularized tumors and natural migration to sites of inflammation, particularly in the central nervous system, and (ii) potent yet controversial antitumor properties through the downregulation of Akt, NFκB, and Wnt signaling pathways to inhibit tumor growth.<sup>34</sup> Supporting this idea, MSCs have been developed as delivery vehicles for interferons, interleukins, prodrugs, oncolytic viruses, antiangiogenic agents, proapoptotic proteins, and growth factor antagonists.<sup>34</sup>

MSCs may be derived from a range of organs but are primarily obtained from bone marrow.<sup>34,150</sup> Bone-marrow-derived MSCs display the largest degree of lineage plasticity, but can only be obtained through invasive procedures at low yields and purities.<sup>34</sup> Nonetheless, marrow-derived cells have been shown to be expanded up to 50 passages over a 10 week period.<sup>34</sup> Two alternate, less-used sources of MSCs have also gained considerable attention: adipose tissue and umbilical cord blood. MSCs can be reliably extracted from subcutaneous tissue and exhibit a nearly identical expansion potential, differentiation capacity, and immunophenotype to marrow-derived MSCs.<sup>34</sup> Genetic stability, easy collection and shipment from the laboratory to the clinic compared to other cell types, and high expansion capabilities enable MSCs to be a potential clinical therapeutic option.<sup>150</sup>

MSCs that express and secrete TRAIL are a potentially promising therapeutic strategy for cancer treatment, and can be engineered to do so *via* (i) viral delivery of a TRAIL payload or (ii) nonviral gene delivery using NPs (Figure 9). Viral-based methods have been discussed in detail previously, and vary in terms of their selectivity, immunogenicity, toxicity, and ability to deliver large payloads.<sup>151–156</sup> In terms on NP-based delivery, a nonviral vector consisting of a low molecular weight PEI and  $\beta$ -cyclodextrin conjugate was complexed with TRAIL-encoding plasmid DNA for delivery to MSCs.<sup>157</sup> Engineered MSCs administered intravenously were shown to migrate into metastatic lung tumors in mice, and induced TRAIL-mediated apoptosis *in vivo* with negligible lung or liver toxicity.<sup>157</sup> In a similar approach, TRAIL-expressing human adipose-derived MSCs were generated through transfection with nucleofector for the treatment of glioma, which extended survival in a rat glioma xenograft model compared to treatment with MSCs alone.<sup>158</sup> Other nonviral approaches have shown that pancreas-derived MSCs genetically engineered to express TRAIL are efficient in targeting cells from the same organ, suggesting that MSCs harvested from a particular diseased tissue could be most efficient in targeting that tissue using MSC-based therapies.<sup>159</sup>

### Adipose Tissue-Derived Stem Cell Therapies

In addition to MSCs, human adipose tissue-derived stem cells (ADSCs) have also been genetically engineered for cell-based therapies.<sup>160</sup> In contrast of MSCs, ADSCs are a more abundant and easily accessible autologous source of stem cells, have fewer ethical concerns in terms of usage, and have demonstrated more oncogenic resistance than human bone-marrow-derived stem cells.<sup>161</sup> In terms of TRAIL delivery, NPs comprised of amino group-ended poly( $\beta$ -amino ester)s, hydrolytically biodegradable polymers that can condense DNA to form NPs, have been utilized to genetically engineer ADSCs to express TRAIL.<sup>160</sup> Engineered ADSCs administered *via* intracranial injection induced significant inhibition of tumor growth and extended survival in a mouse intracranial xenograft model of glioblastoma multiforme.<sup>160</sup> This cell-based approach could represent an alternative therapeutic approach for malignant brain tumors that cannot be removed *via* surgery.

The translation of stem cell-based therapies into viable clinical strategies is challenged by the fact that large fractions of systemically infused stem cells become entrapped within the lung due to both their large size and *via* receptor–ligand mediated adhesion to the vessel wall. Such interactions prevent a large number of stem cells from homing and migrating to diseased tissue.<sup>162</sup> Furthermore, the site of stem cell administration affects the route by which cells reach target organs. For instance, locally administered stem cells often do not survive as a result of a nutrient and oxygen diffusion limitations.<sup>163</sup> In addition to entrapment, stem cells may not survive due to conditions in circulation (*i.e.*, blood vessel diameter, cell deformability).<sup>163</sup> Thus, further research to reduce stem cell entrapment and enhance homing is needed to advance cell-mediated TRAIL therapies. Additionally, the heterogeneity of various stem cell populations and mixed results of cell-based therapies in clinical trials for engraftment to tissues have challenged their translational potential.<sup>162</sup> Nonetheless, through the optimization of such delivery techniques and a stronger understanding of their homing and migration, tumor-tropic stem cell-based therapies can be leveraged for site-specific delivery and secretion of TRAIL to target tumor cells.

## Nanoconjugates for TRAIL Delivery

Beyond NPs, nanoscale conjugates consisting of a therapeutic protein bound to PEG or HSA have also been designed to enhance drug delivery to tumors. Conjugates offer several benefits to delivery, including enhanced TRAIL pharmacokinetics, are nontoxic, nonimmunogenic, and are highly soluble in water.<sup>164</sup> Conjugates are ideal delivery vehicles in terms of their size in that they are generally smaller than most lipid and polymeric NPs, which are taken up by the mononuclear phagocytic system in the liver and spleen.<sup>165</sup> They are also typically large enough to avoid clearance by the kidney, which excretes particles smaller than 5.5 nm in hydrodynamic diameter.<sup>165,166</sup>

### PEG-Based TRAIL Conjugates

PEG is widely used to enhance the circulation half-life of therapeutics in the bloodstream.<sup>167</sup> Conjugation of PEG to TRAIL through PEGylation techniques prolongs *in vivo* pharmacokinetics by increasing molecular size and steric hindrance, extending circulation half-life, and delaying renal excretion.<sup>91</sup> In a recent study, PEGylated TRAIL conjugates were synthesized with varying PEG molecular weights (2–30 kDa), and increased PEG molecular weight was found to improve circulation half-life to 17.8 h compared to 1.1 h for soluble TRAIL protein.<sup>168</sup> Larger PEG-TRAIL conjugates also significantly reduced overall tumor burden in a mouse colon cancer xenograft model compared to smaller conjugates and soluble TRAIL protein.<sup>168</sup> In addition to extended circulation half-life, PEG can be further customized to enhance both bioactivity and targeting. Multifunctional TRAIL conjugates have been designed by incorporating a zipper sequence to facilitate TRAIL trimerization and enhance potency, while also adding PEG to the N-terminus of TRAIL to increase circulation half-life.<sup>169</sup> Heterobifunctional PEGs have also been employed to conjugate both TRAIL and the tumor-targeting ligand transferrin to enhance delivery.<sup>91</sup>

While TRAIL-PEG conjugates are potentially promising delivery systems, the steric effect of a larger PEG molecule could impact the bioactivity of PEGylated TRAIL conjugates.<sup>168</sup> Thus, while optimized PEGylated TRAIL constructs enhance circulation half-life, effects such as steric hindrance can limit its biological activity and thus should be considered when designing conjugates. PEGylation can also occur at random molecular positions, thus affecting biological activity.<sup>167</sup>

### HSA-Based TRAIL Conjugates

As an alternative to PEG, circulating serum proteins such as HSA have been explored to enhance the half-life of TRAIL. The conjugation of albumin protein to therapeutics is a common strategy for extending circulation half-life and has been applied to interferon alpha, erythropoietin, coagulation factors IX and VIIa, human growth hormone, and TNF.<sup>170</sup> Noting that serum albumin is the most abundant protein in blood plasma, the protein can be covalently conjugated to a therapeutic of interest or be allowed to noncovalently interact endogenously.<sup>170,171</sup> In the case of TNF ligands, albumin proteins such as HSA reduce clearance from circulation yet do not interfere with biological activity.<sup>172</sup> Conjugation of HSA to the N-terminus of TRAIL has been shown to increase circulation half-life from 1 to 15 h, with significantly reduced dosages shown to achieve a significant *in vivo* antitumoral

effect in comparison with unconjugated TRAIL.<sup>172</sup> HSA-TRAIL conjugates have also demonstrated preserved bioactivity in addition to an improved pharmacodynamics profile.<sup>173</sup> In comparison with PEG, HSA-TRAIL conjugates have been understudied and thus warrant further investigation.

## CONCLUSIONS

The ability of TRAIL to induce cell death specifically in cancer cells without inducing normal cell toxicity renders the immune cytokine as a promising therapeutic for a wide range of cancers. Recent innovations in the development of polymers, lipids, and inorganic materials are being used to formulate NPs to overcome the challenges faced by TRAIL-based therapeutics in clinical trials, including the improvement of TRAIL circulation half-life, targeting, and bioactivity. In addition to the delivery of TRAIL therapeutic proteins, advances in NPbased systems have also enabled the delivery of TRAIL-encoding plasmid DNA *in vivo*, opening up therapeutic strategies to transfect tumor cells and the surrounding microenvironment for TRAIL secretion locally at target tumor sites. NPs have also been employed in cell-based therapies, including *ex vivo* genetic engineering of mesenchymal stem cells, which can traverse complex biological barriers in the body to migrate into tumors and secrete therapeutic TRAIL proteins. While the range of NP-based approaches reviewed here show promise in overcoming many hurdles, additional challenges remain in the development of effective TRAIL cancer therapeutics.

TRAIL resistance remains a significant barrier to clinical translation, as certain types of tumor cells overexpress intracellular proteins, including cFLIP, inhibitor of apoptosis proteins (IAPs) and antiapoptotic members of the Bcl-2 family.<sup>174</sup> Moreover, recent research suggests that TRAIL death receptor expression induces side effects in addition to apoptosis, such as cancer cell invasion, which can pose challenges in the context of cancer therapy.<sup>175</sup> Although cancer cells show different levels of sensitivity to TRAIL, a wide range of agents have been reported which sensitize cancer cells, including low doses of conventional chemotherapeutics (doxorubicin, 5-fluorouracil, irinotecan, paclitaxel, camptothecin, cisplatin), Bcl-2 inhibitors, IAP antagonists, proteasome inhibitors, histone deacetylase inhibitors, CD20 antibodies, irradiation, aspirin, as well as natural products including piperlongumine, resveratrol, and curcumin.<sup>9,176</sup> Given that the majority of these sensitization agents can be encapsulated or conjugated to TRAIL NPs highlighted in this review, we envision numerous opportunities in future research to design NPs for TRAIL combination therapies to overcome drug resistance issues faced in clinical studies.

Moving forward, these delivery materials must be further assessed in advanced animal models, such genetically engineered mouse models of cancer, which better mimic the heterogeneity of human cancers encountered in the clinic. Major differences exist between mice and humans, as mice only express one death receptor (DR5), which shares 43% and 49% sequence homology with human TRAIL-R1 and TRAIL-R2.<sup>177,178</sup> Mice also express two decoy receptors that are distinct from human decoy receptors.<sup>177,179</sup> While promising, the optimal delivery process needed to improve TRAIL therapy in the clinic remains unsolved, and there is significant room for innovation and development of NPs to achieve these goals.



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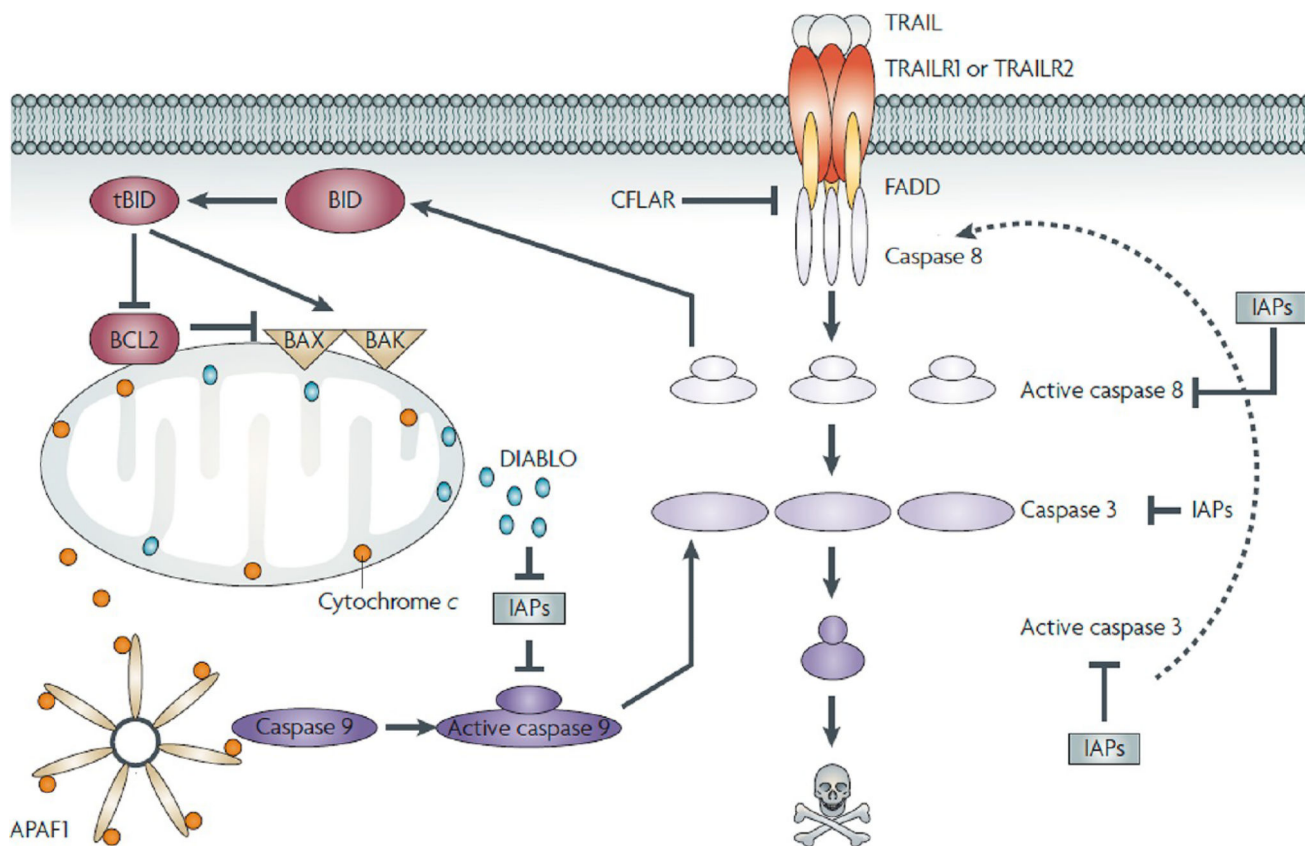
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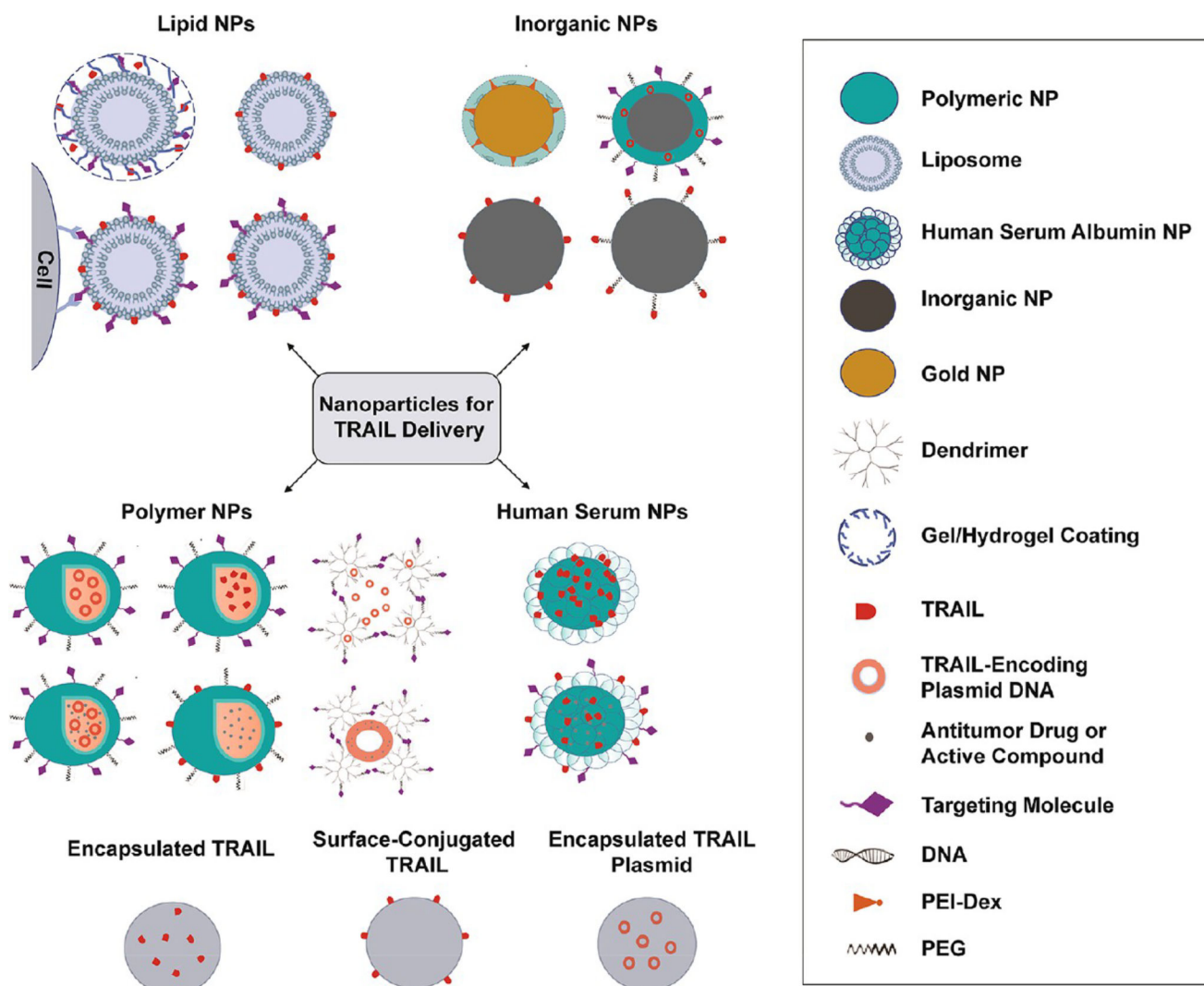
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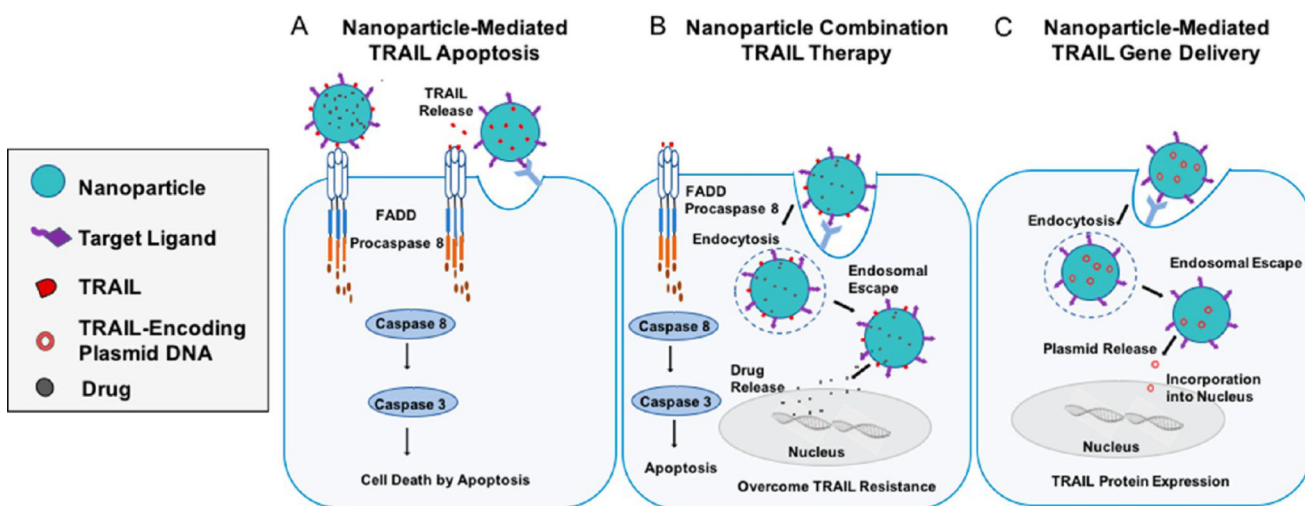


**Figure 1.**

Apoptotic signaling through the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) pathway. Binding of TRAIL to TRAIL receptor 1 (TRAILR1 or D4) or TRAILR2/DR5 results in receptor oligomerization and recruitment of FAS-associated protein with death domain (FADD) and caspase 8 to form a functional death-inducing signaling complex (DISC). Upon DISC formation, caspase 8 is cleaved and activated, which in turn can cleave and activate caspase 3 and the BH3-only protein BID. Active, cleaved BID (tBID) can bind to pro-apoptotic Bax and Bak, resulting in mitochondrial membrane permeabilization and release of mitochondrial proteins cytochrome *c* and DIABLO. Cytochrome *c*, apoptotic protease-activating factor 1 (APAF1), and caspase 9 combine with ATP to form a functional apoptosome that results in cleavage and activation of caspase 9, which can then cleave caspase 3. DIABLO suppresses the caspase-inhibitory activities of inhibitor of apoptosis proteins. Caspase 3 can cleave a large number of intracellular targets, resulting in the morphological and biochemical hallmarks of apoptosis. Caspase 3 can also cleave and activate caspase 8, thereby amplifying the apoptotic signal. Reprinted with permission from ref 4. Copyright 2016 Nature.

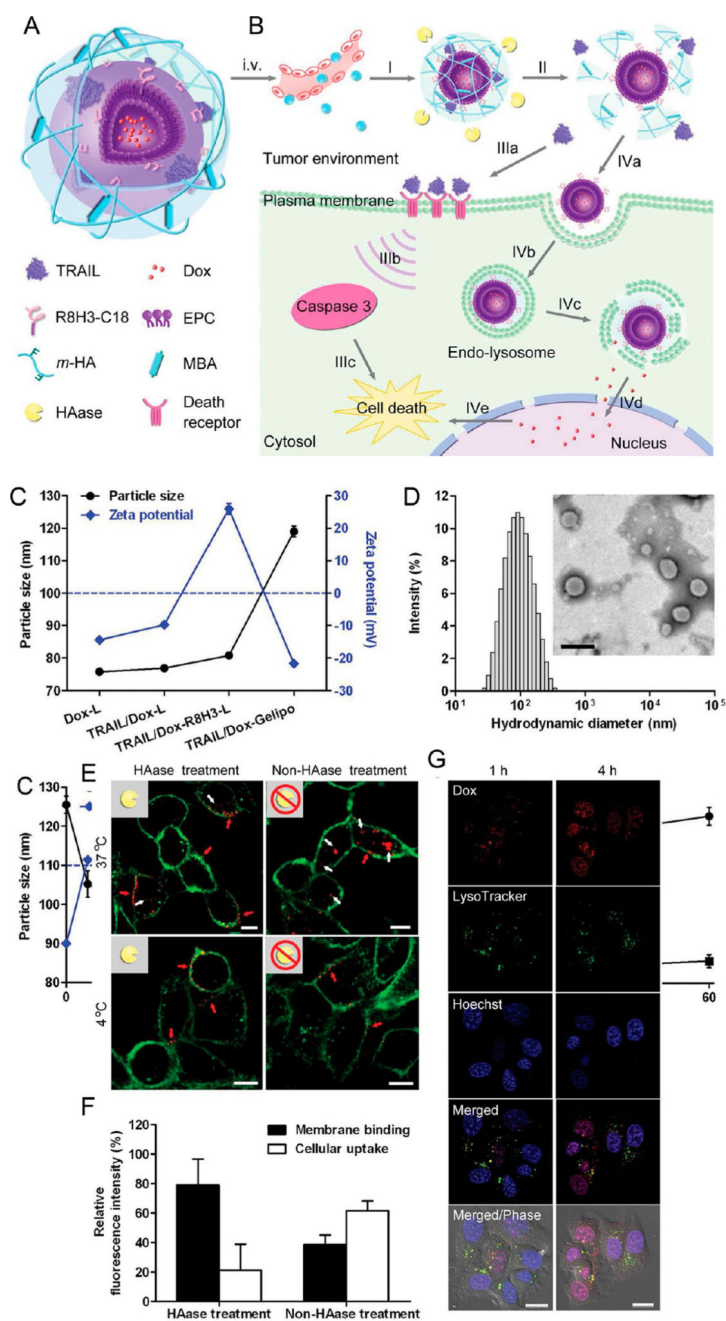


**Figure 2.**  
Examples of classes of NPs used for TRAIL-based cancer therapy.



**Figure 3.**

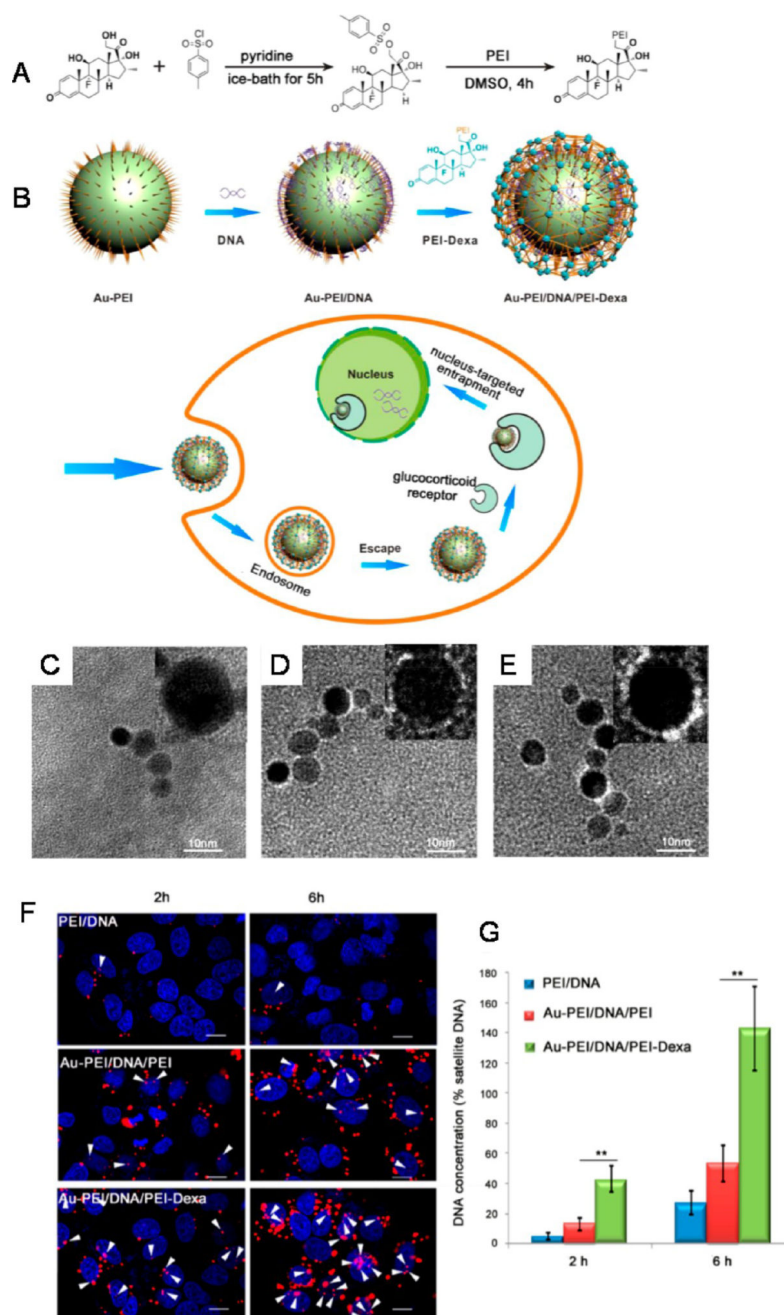
Approaches to utilize NPs to induce TRAIL-mediated apoptosis in tumor cells *via* (A) TRAIL-coated NPs binding to the tumor cell surface, (B) a combination NP-based approach that delivers encapsulated small molecule drugs to sensitize tumor cells to TRAIL-mediated apoptosis, or (C) gene delivery to cells to induce secretion of TRAIL.



**Figure 4.** Schematic design of TRAIL/Dox-Gelipo for sequential and site-specific drug delivery. (A) Main components of TRAIL/Dox-Gelipo: R8H3-modified liposomal core loading doxorubicin (Dox) and cross-linked hyaluronic acid (HA) gel-based outer shell encapsulating TRAIL. (B) Sequential delivery of TRAIL to the plasma membrane and Dox to the nuclei by TRAIL/Dox-Gelipo for combination cancer treatment. I, accumulation of Gelipo (blue balls) at the tumor site through the passive and active targeting effects; II, degradation of HA cross-linked shell by HAase; IIIa, released TRAIL binding onto the death receptors on the plasma membrane; IIIb, activation of the caspase 3 signaling pathway; IIIc, Endo-lysosome formation; IVa-IVd, Dox nuclear uptake and cell death; IVe, Cell death. (C) Particle size and zeta potential for various formulations. (D) Hydrodynamic diameter distribution histogram and TEM image. (E) Particle size vs temperature (0, 37°C) for HAase and non-HAase treatments. (F) Bar graph of relative fluorescence intensity for membrane binding and cellular uptake. (G) Fluorescence microscopy images of Dox, LysoTracker, Hoechst, and merged images at 1h and 4h.

induction of the cell death; IVa, exposure of R8H3 facilitating the tumor cellular uptake of Dox-R8H3-L; IVb, internalization of Dox-R8H3-L into the tumor cells; IVc, endolysosomal escape; IVd, accumulation of the released Dox into nucleus; IVe, intercalation of Dox on DNA inducing the cell death. (C) Particle size and  $\zeta$ -potential of Dox-L, TRAIL/Dox-L, TRAIL/Dox-R8H3-L, and TRAIL/Dox-Gelipo. (D) Hydrodynamic size of TRAIL/Dox-Gelipo measured by dynamic light scattering. Inset: TEM image of TRAIL/Dox-Gelipo. Scale bar is 200 nm. (E) Membrane binding efficiency of r-TRAIL from r-TRAIL-Gelipo with and without 30 min of HAase pretreatment on MDA-MB-231 cells observed by confocal laser scanning microscopy (CLSM). The plasma membranes were stained by Alexa Fluor 488 conjugate of wheat germ agglutinin. Scale bars are 10  $\mu$ m. (F) Quantitative analysis on the r-TRAIL amount on the plasma membrane and in the cells. (G) Intracellular delivery of TRAIL/Dox-Gelipo after 30 min of HAase pretreatment on MDA-MB-231 cells at different time observed by CLSM. The late endosomes and lysosomes were stained by LysoTracker Green, and the nuclei were stained by Hoechst 33342. Scale bars are 20  $\mu$ m. Reprinted with permission from ref 56. Copyright 2014 Wiley.





**Figure 5.** (A) Schematic illustration of PEI-Dexa synthesis and (B) nucleus-targeted sandwich-type Au-PEI/DNA/PEI-Dexa complex for intercellular DNA delivery. Transmission electron microscopic images of (C) Au-PEI, (D) Au-PEI/DNA, and (E) Au-PEI/DNA/PEI-Dexa. (Insets) Higher magnification micrographs of the assembled NPs. (F) Intracellular localization of PEI/DNA, Au-PEI/DNA/PEI (w/w/w, 0.6/1/2), and Au-PEI/DNA/PEI-Dexa (w/w/w, 0.6/1/2) in Hep3B cells after gene transfection for 2 or 6 h (red, Cy5-labeled pDNA; blue, DAPI stained cell nuclei; pink, colocalization). A dose of 1  $\mu\text{g}$ /well of Cy5-labeled pDNA was used. White arrows show colocalization of nucleus and plasmid. Bars = 15  $\mu\text{m}$ .

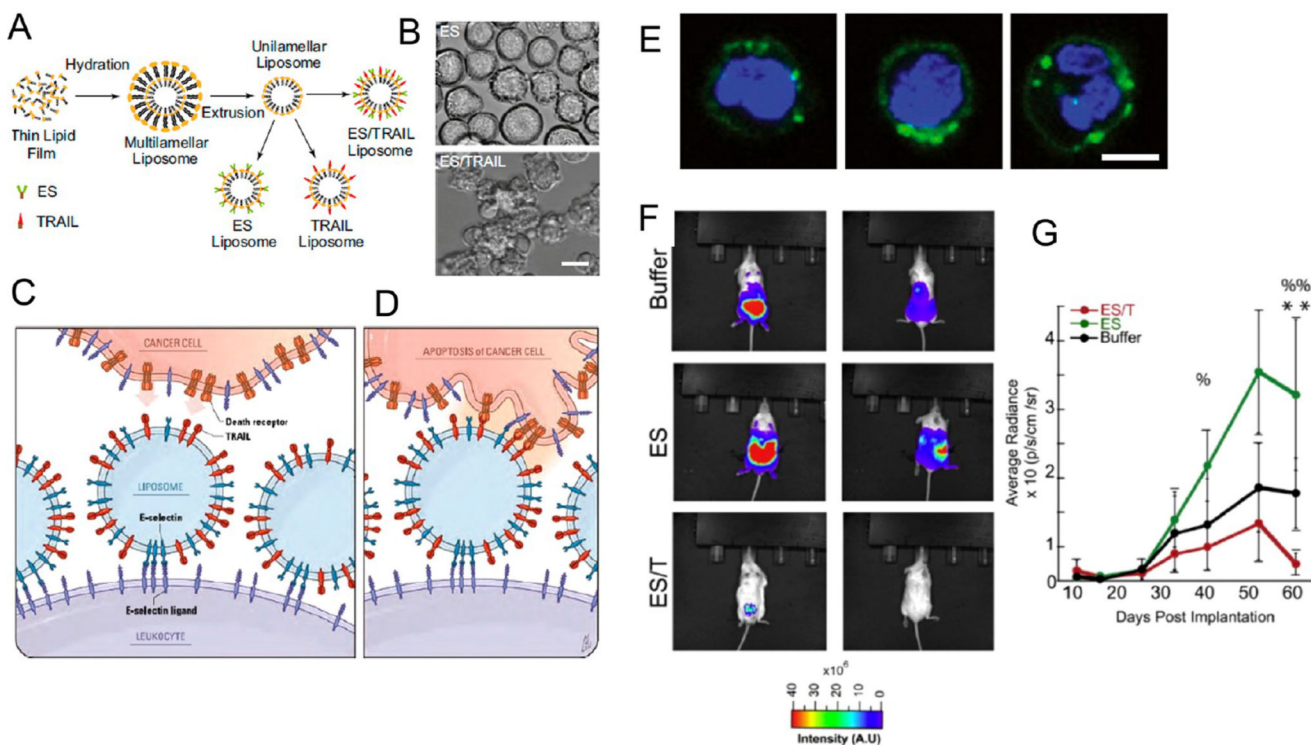
(G) Intranuclear concentration of pFlag-cmv2 in Hep3B cells treated by PEI/DNA, Au-PEI/DNA/PEI (w/w/w, 0.6/1/2), and Au-PEI/DNA/PEI-Dexa (w/w/w, 0.6/1/2) for 2 or 6 h. Nuclear sections were purified by nucleocytoplasmic separation and examined Flag-cmv2 relative concentration to human satellite DNA, a good control for cellular DNA detection. Reprinted with from ref 127. Copyright 2014 American Chemical Society.

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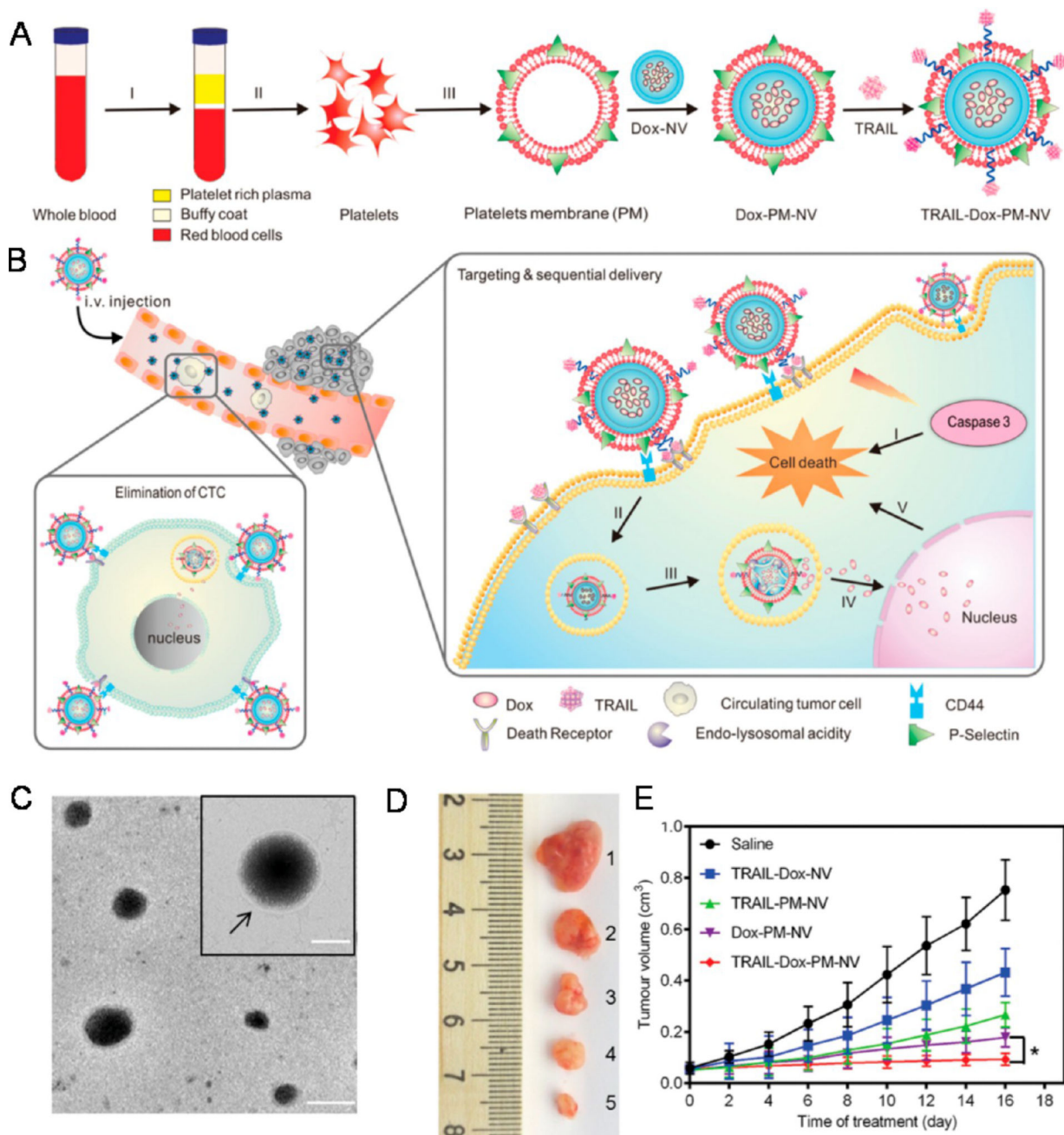
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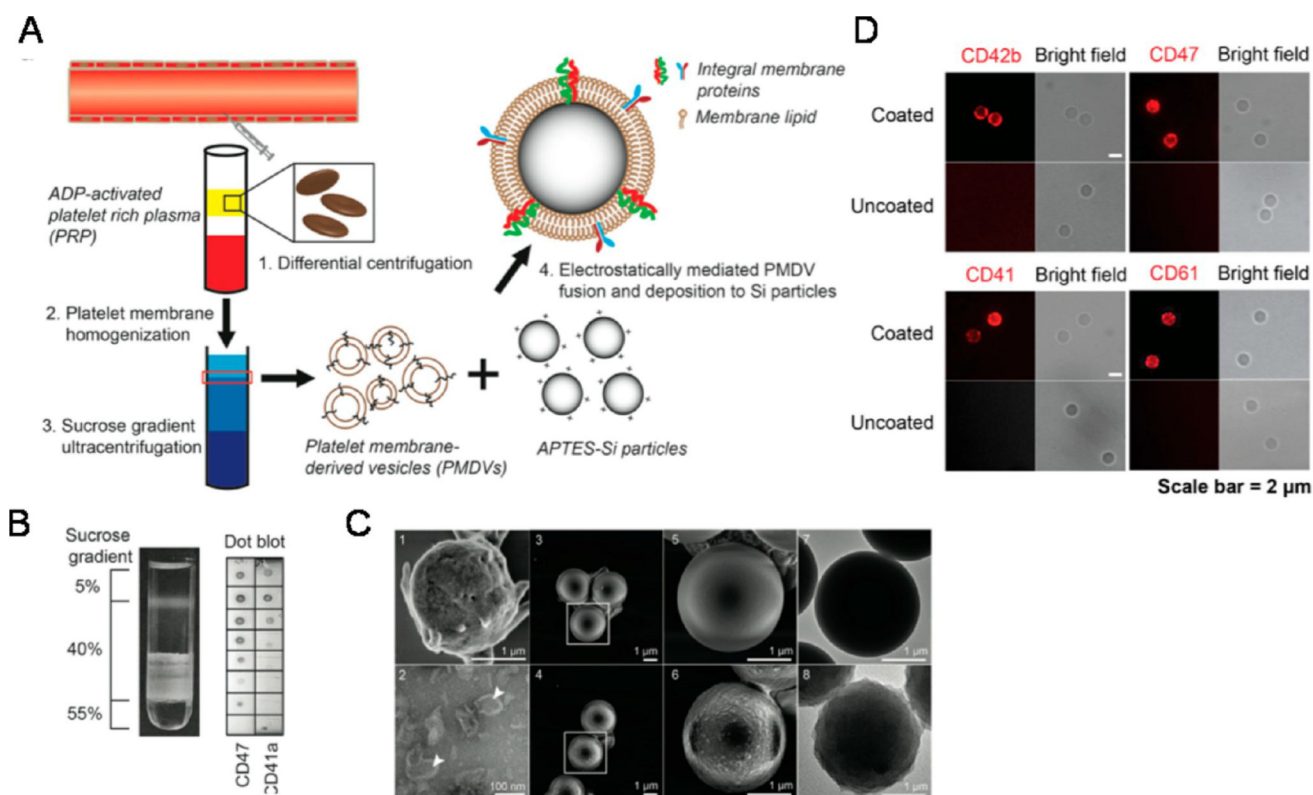
**Figure 6.**

ES/TRAIL liposomes adhesively interact with and kill cancer cells under uniform shear flow. (A) Synthesis of ES, TRAIL, and ES/TRAIL unilamellar liposomes using a thin film hydration method. Briefly, lipids in chloroform were dried overnight to form a thin lipid film. Lipids were then hydrated and subjected to freeze–thaw cycles to form multilamellar liposomes, which were extruded through membranes to form unilamellar liposomes. ES, TRAIL, or a combination of ES and TRAIL was then conjugated to Ni-NTA on the liposome surface. To assess the ability of ES/TRAIL liposomes to target and kill cancer cells under flow, ES/TRAIL liposomes were added to a suspension of COLO205 cancer cells and exposed to shear flow in a cone-and-plate viscometer at a shear rate of  $188 \text{ s}^{-1}$  for 2 h. Cells were then removed, washed, placed into culture for 24 h, and assessed for cell viability. (B) COLO 205 morphology after treatment with ES (top) and ES/TRAIL (bottom) liposomes under shear flow (Scale bar,  $20 \mu\text{m}$ ). (C,D) Schematic of the two-step mechanism involving decoration of leukocytes with liposomes (C), which then contact circulating cancer cells and activate the death receptor (D). (E) Confocal images of ES/TRAIL liposomes (green) bound to human leukocytes (blue, cell nuclei) after exposure to shear flow in whole blood in a cone-and-plate viscometer at  $188 \text{ s}^{-1}$  for 30 min. Leukocytes have nuclear morphology characteristic of monocytes (left), lymphocytes (center), and neutrophils (right) (scale bar,  $5 \mu\text{m}$ ). (F) Whole animal BLI of the ventral (left) and dorsal (right) side of representative animals from each treatment group at the end of the trial (week 9). (G) Average radiance from the primary tumor. Comparisons were made *via* one-way ANOVA with Tukey's post-test. Error bars represent the mean  $\pm$  SD at each time point. ES vs ES/TRAIL: % $p < 0.05$ , %% $p < 0.01$ . Buffer vs ES/TRAIL: \*\* $p < 0.05$ . Reprinted with permission from refs 139 and 140. Copyright 2014 National Academy of Sciences and 2016 Elsevier.

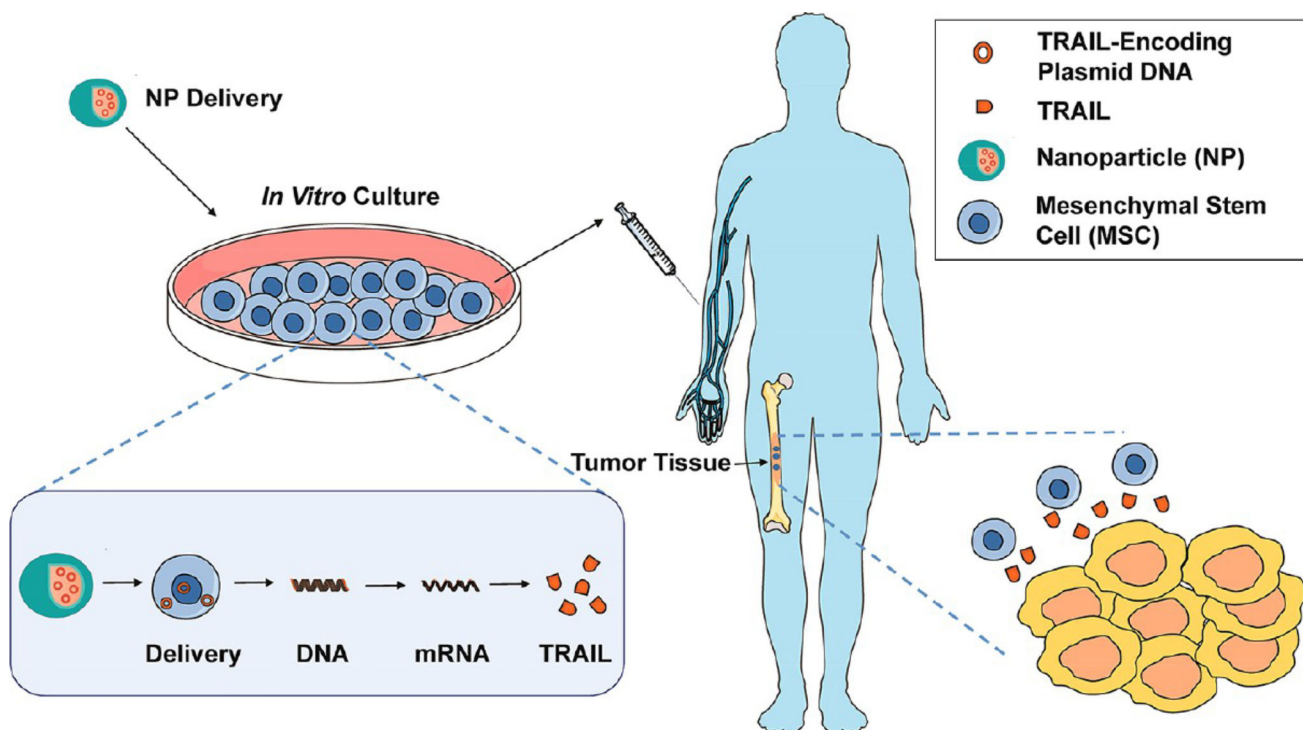


**Figure 7.** Schematic design of a drug-loaded platelet membrane nanovehicle (PM-NV) for targeting and sequential drug delivery. (A) Main components of TRAIL-Dox-PM-NV: TRAIL-conjugated PM derived from platelets; Dox-NV. (I) centrifugation of whole blood; (II) isolation of platelets; (III) extraction of PM. (B) *In vivo* elimination of CTCs and sequential delivery of TRAIL and Dox. TRAIL-Dox-PM-NV captured the CTCs *via* specific affinity of P-selectin and overexpressed CD44 receptors and subsequently triggered TRAIL/Dox-induced apoptosis signaling pathways. (I) Interaction of TRAIL and death receptors (DRs) to trigger the apoptosis signaling; (II) internalization of TRAIL-Dox-PM-NV; (III)

dissociation of TRAIL-Dox-PM-NV mediated by the acidity of lyso-endosome; (IV) release and accumulation of Dox in the nuclei; (V) intrinsic apoptosis triggered by Dox. (C) Transmission electron microscope image and hydrodynamic size distribution of PM-NV. Arrow indicates the existence of PM. The scale bar: 100 nm; inset: 50 nm. (D) Representative images of the MDA-MB-231 tumors after treatment with different TRAIL/Dox formulations at day 16 (from top to bottom: (1) saline, (2) TRAIL-Dox-NV, (3) TRAIL-PM-NV, (4) Dox-PM-NV, (5) TRAIL-Dox-PM-NV) at TRAIL dose of  $1 \text{ mg kg}^{-1}$  and Dox dose of  $2 \text{ mg kg}^{-1}$ . (E) MDA-MB-231 tumor growth curves after intravenous injection of different TRAIL/Dox formulations. Error bars indicate SD ( $n = 5$ );  $*p < 0.05$  (two-tailed Student's  $t$ -test). Reprinted with permission from ref 148. Copyright 2015 Wiley.



**Figure 8.** Functionalization and characterization of PMDV-coated Si particles. (A) Schematic of preparing platelet membrane-coated Si particles. (B) Detection of membrane protein associated lipid layer in discontinuous sucrose gradient solution by dot blot assay. Lipid fractions were identified as translucent layers in between two sucrose concentrations. (C) SEM and TEM characterization. SEM images: (1) activated platelets, (3,5) APTES-Si particles, (4,6) PMDV-coated Si particles. TEM images: (2) PMDVs, (7) APTES-Si particles, (8) PMDV-coated Si particles. Arrow head identifies the hollow structure of PMDVs. (D) Immunofluorescence staining of CD42b, CD47, CD41, and CD61 in PMDV-coated or uncoated Si particles. Far-red and bright-field images are presented. Reprinted with permission from ref 149. Copyright 2015 Elsevier.



**Figure 9.** Cell-based therapies for TRAIL-based cancer therapeutics. MSCs can be transfected by either viral or nonviral delivery materials, such as NPs, to express and/or secrete TRAIL. MSCs can then cross biological barriers and deliver TRAIL to induce apoptosis in tumor cells.

**Table 1**

Examples of TRAIL-Based Therapeutic Approaches in Clinical Trials

| approach                                       | details   | tolerability                  | ref    |
|--|---|-------------------------------|--------|
| <b>recombinant human soluble TRAIL protein</b> |   |                               |        |
| Dulanermin                                     | RhApo2L/TRAIL aa. 114–281   | safe                          | 7,8,11 |
| CPT  | circularly permuted TRAIL; N-terminus of Apo2L/TRAIL aa. 121–135 is lined to C-terminus of the aa. 135–281 of Apo2L/TRAIL | safe                          | 12     |
| <b>agonistic TRAIL receptor antibodies</b>     |   |                               |        |
| Mapatumumab                                    | fully human agonistic monoclonal antibody against DR4   | safe                          | 13–17  |
| Lexatumumab                                    | fully human agonistic monoclonal antibody against DR5   | safe                          | 18     |
| Conatumumab                                    | fully human agonistic monoclonal antibody against DR5   | safe                          | 19–21  |
| Drozitumab                                     | fully human agonistic monoclonal antibody against DR5   | safe                          | 22,23  |
| Tigatuzumab                                    | humanized agonistic monoclonal antibody against DR5   | safe                          | 24–27  |
| TAS266   | tetrameric agonist nanobody against DR5   | induces hepatotoxicity        | 28     |
| <b>other</b>                                   |   |                               |        |
| T-cell-based immunotherapy                     | autologous T cells transduced with a T cell receptor recognizing TRAIL bound to DR4                                       | terminated; no results posted |        |