8.1 Nanomedicine

8.1.1 Polymeric nanoparticles

Polymeric nanoparticles comprise synthetic polymers which allow alteration of many properties such as biodegradability, molecular weight and hydrophilicity. Various methods have been developed to synthesize polymeric nanoparticles like nanoprecipitation, solvent diffusion/displacement, emulsification, nanospray etc (Li et al., 2017, Tran et al., 2017). Typical polymeric nanoparticles are dense matrix systems with predictable degradation curves which allow easy manipulation of drug release from these systems. However, there are certain limitations with these systems such as limited shape and large size distribution. These nanoparticles are usually spherical, although a various sizes might be developed at the time of synthesis utilizing novel techniques (Joshi et al., 2010, Kulkarni and Feng, 2013, Vuddanda et al., 2015). Nowadays, PRINT approach, which utilizes particle replication in non-wetting templates, allows development of uniform nanoparticles hence easy customization of particle size and shape (National Center for Biotechnology Information. Pubchem Compound Database; Cid=45055483).

8.1.2 Liposomal nanoparticles

Liposomes are spherical nanoparticles developed using lipid bilayers. These nanoparticles are prepared after addition of amphiphilic lipid and water or hydrophilic liquid (Li et al., 2017, Tran et al., 2017). This depicts size range of 50-500 nm. This procedure also allows the encapsulation of hydrophilic drugs by simply dissolving the drug in the hydrophilic liquid utilized for preparation. Hydrophobic drugs can also be encapsulated by adding into lipid solution and the drug resides in the lipid bilayer. There are several methods utilized for

preparation of liposomes like sonication, extrusion, solvent injection and reverse phase evaporation. These nanocarriers can be widely used for targeted drug delivery (Vijayakumar et al., 2016b, Vijayakumar et al., 2016c, Zhigaltsev et al., 2005).

8.1.3 Protein‑drug conjugated nanoparticles

In this type of nanoparticles proteins are directly conjugated to drug. The conjugation link is biodegradable in *in-vivo* conditions. The biodegradable linker is destroyed by several enzymes present in the body. This can lead to premature drug release. However, such protein drug conjugates which stay in place and release the drug after reaching target site can overcome this barrier. Hence more precise and controllable delivery with lesser toxic effects can be achieved. These nanoparticles have size nearly 10 nm which further enhance half life of drugs. Recently, antibodies are also employed to improve targeting of protein drug conjugated nanoparticles (Tran et al., 2017).

8.1.4 Dendrimeric nanoparticles

These nanoparicles comprises an exclusive class of polymeric macromolecules occurred naturally. Dendrimers are spherical macromolecules having many branches which originate from the central point. These are created layer by layer; the initial core in incorporated onto previour layer then branching is allowed to take place. The size and degree of branching can be controlled utilizing specific initiator cores. Controlled size and branching further minimize the polydispersity of nanoparticles. Careful setting up the cores and branching units will help to specify various properties of these nanoparticles like size, branch density, molecular weight, flexibility and water solubility. The branches can be decorated utilizing a range of molecule for entrapment and further release of drugs (Li et al., 2017, Tran et al., 2017).

8.1.5 Micellar nanoparticles

These nanoparticles acquire a core-shell structure. The shell can be hydrophilic e.g. by using PEG or hydrophobic by utilizing Poly lactic acid (PLA), Poly (lacti-co-glycolic acid) (PLGA), polystyrene, poly (cyanoacrylate), poly (vinyl pyrrolidone) (PVP), and polycaprolactone (PCL). These copolymers are biodegradable, biocompatible and possess the ability to entrap hydrophobic molecules. E.g. paclitaxel loaded mPEG-PLA (Genexol-PM) is FDA approved (Li et al., 2017, Tran et al., 2017).

These nanoparticles can be obtained by self-assembly of amphiphilic copolymers in aqueous environment once the critical micelle concentration (CMC) is reached. The core is hydrophobic and can encapsulate hydrophobic moieties while the hydrophilic shell entraps the hydrophilic moieties. Thus the hydrophilic shell provides aqueous solubility and steric solubility to the miceller nanoparticles. Using miceller nanoparticles, drugs can be covered by a water soluble layer, which in turn enhance the hydrophilicity and bioavailability of poorly soluble drugs. Moreover, the hydrophilic shell provides protection and enhances *in-vivo* circulation (Wang et al., 2015). Recently, many nanomiceller drugs gain success to reach clinical trials and market. These miceller nanoparticles can be further decorated with targeting moieties to achieve active targeting (Agrawal et al., 2017a).

8.1.6 Other nanoparticle platforms

8.1.6.1 Inorganic, metallic nanoparticles:

Gold has been extensively used for theranostic purpose i.e. therapy and diagnosis of cancer with or without drug loading. Gold depicts strong optical absorbance by virtue of this property it can be used diagnosis. Moreover, its photothermal properties render it suitable for anticancer therapy. Nanoparticles may be fabricated with gold as complex structures, which

in turn enhance the efficiency of drug release. E.g. drugs may be conjugated with the gold nanoparticle surface or structures containing hollow cores may be developed to enhance encapsulation efficiency (Li et al., 2017, Tran et al., 2017). Many of these structures can be easily created and specifically designed, such as to include a wide range of optical properties. Moreover, various modifications can be incorporated to further modify the release of drugs from such nanoparticles. Such as adding layers of thermoresponsive polymers. Further, photothermal properties can be combined with thermoresponsive polymers whereby shining a laser to heat gold nanopaticles when they are near tumor site can control the drug release as well as minimized the non specific toxicity (Huang and El-Sayed, 2010, Jain et al., 2012).

8.1.6.2 Carbon nanotubes

Carbon nanoparticles are basically tubes made of carbon with diameters in nanorange These carbon nanotubes have been widely utilized for cancer therapy. These can bind to a wide variety of various biological molecules and penetrate the cells through endocytosis (Li et al., 2017, Tran et al., 2017). Single walled carbon nanotubes (SWCNTs) are utilized to prepared suspensions in physiological medium which are highly stable and can be used in biological environments. These SWCNTs can also attach various molecules through cleavable disulfide bonds, which allow release of drugs *in-vivo* by action of enzymes. Recently these carbon nanotubes are also shown to be promising for treatment utilizing their optical properties. E.g. carbon nanotubes can be used for phototherma and phothodynamic therapy where they can damage cancer cells photochemically (Singh et al., 2016).

8.1.6.3 Silver nanoparticles:

Silver nanoparticles also emerged as a potential tool for treatment of cancer. Although, the exact mechanism is not defined, silver is supposed to react with acidic environment of tumor

cells and generates reactive oxygen species (ROS). These ROS damages the tumor cells leading to apoptosis. Silver also possess anti-angiogenic properties and was found to inhibit vascular endothelial growth factor (VEGF) (Li et al., 2017, Tran et al., 2017). However, the toxicity associated with the silver is main concern. The same can be overcome by creating nanoparticles with biocompatible shell which can degrade in specific environments. Moreover, this layer also provides platform for conjugation of various ligands. Further, the toxicity concern required to be addressed using *in- vivo* animal models (Pugazhendhi et al., 2018, Yuan et al., 2017).

8.2 Research Envisaged

Vinorelbine bitartrate (VRL), a semi-synthetic vinca alkaloid exhibits anticancer efficacy against various tumors. It is primarily indicated for the treatment of breast cancer and NSCLC as a first line therapy or in combination with other chemotherapeutic agents. Though VRL is better tolerated among all vinca alkaloids it causes toxicities like neutropenia (main dose-limiting toxicity), nausea, vomiting, diarrhoea, constipation, alopecia and peripheral neuropathy. It is a vesicant and causes injection site reaction, superficial phlebitis accompanied by erythema, pain, vein discoloration and tenderness along the vein(Drummond et al., 2009, Emanuela et al., 2018, Goa and Faulds, 1994, Kreidieh et al., 2016, Vassilomanolakis et al., 2001, Wan et al., 2008, You et al., 2007, Zhang et al., 2011, Zhang et al., 2008).

Moreover, most single drug based anticancer therapies are seldom effective owing to associated multiple genetic alterations and molecular abnormalities. The anticancer efficacies can be greatly improved by utilizing anticancer drug combinations. Drug combinations can

also eliminate the drug resistance owing to non-overlapping mechanisms and can also reduce dose dependent side effects.

The poly-mechanistic RES was supposed to exhibit synergistic activity with VRL on breast cancer cells which will improve the therapeutic efficacy of VRL. The synergism among two drugs can lead to VRL dose reduction which in turn can result in reduced toxicity. However, it is not feasible to analyze all combinations in human subjects so the current state-of-the-art utilizes in vitro synergy rather than anti-proliferative studies on 1-3 tumor cell lines to assess higher levels of anticancer drug combinational paradigms (Chen et al., 2016, Greco and Vicent, 2009, Kashif et al., 2015, Lu et al., 2015).

The major drug related toxicities are dose dependent therefore attempts can be taken to reduce the dose and dosing frequency which can be achieved by combining VRL with a non-toxic drug Resveratrol (RES). RES, a naturally occurring polyphenol compound found in a variety of food sources, is already proved to reduce VRL induced vascular endothelial cell injury by reduction in cellular apoptosis, reactive oxygen species (ROS) generation and superoxide dismutase (SOD) levels (Zhang et al., 2013).RES displayed a wide range of pharmacological properties, such as anticancer, antiplatelet, estrogenic and anti-inflammatory. Although, this relationship can be established easily under *in-vitro* controlled conditions, clinical translation of such information is complicated by the uncoordinated pharmacokinetics of free-drug combinations. The dissimilar or independent pharmacokinetics of individual drugs may lead to exposure of tumor cells to antagonistic or suboptimal ratios with corresponding loss in therapeutic activity (Ashley et al., 2016, Chen et al., 2016, Greco and Vicent, 2009, Kashif et al., 2015, Lu et al., 2015, Mayer et al., 2006, Tardi et al., 2009, Wang et al., 2014).

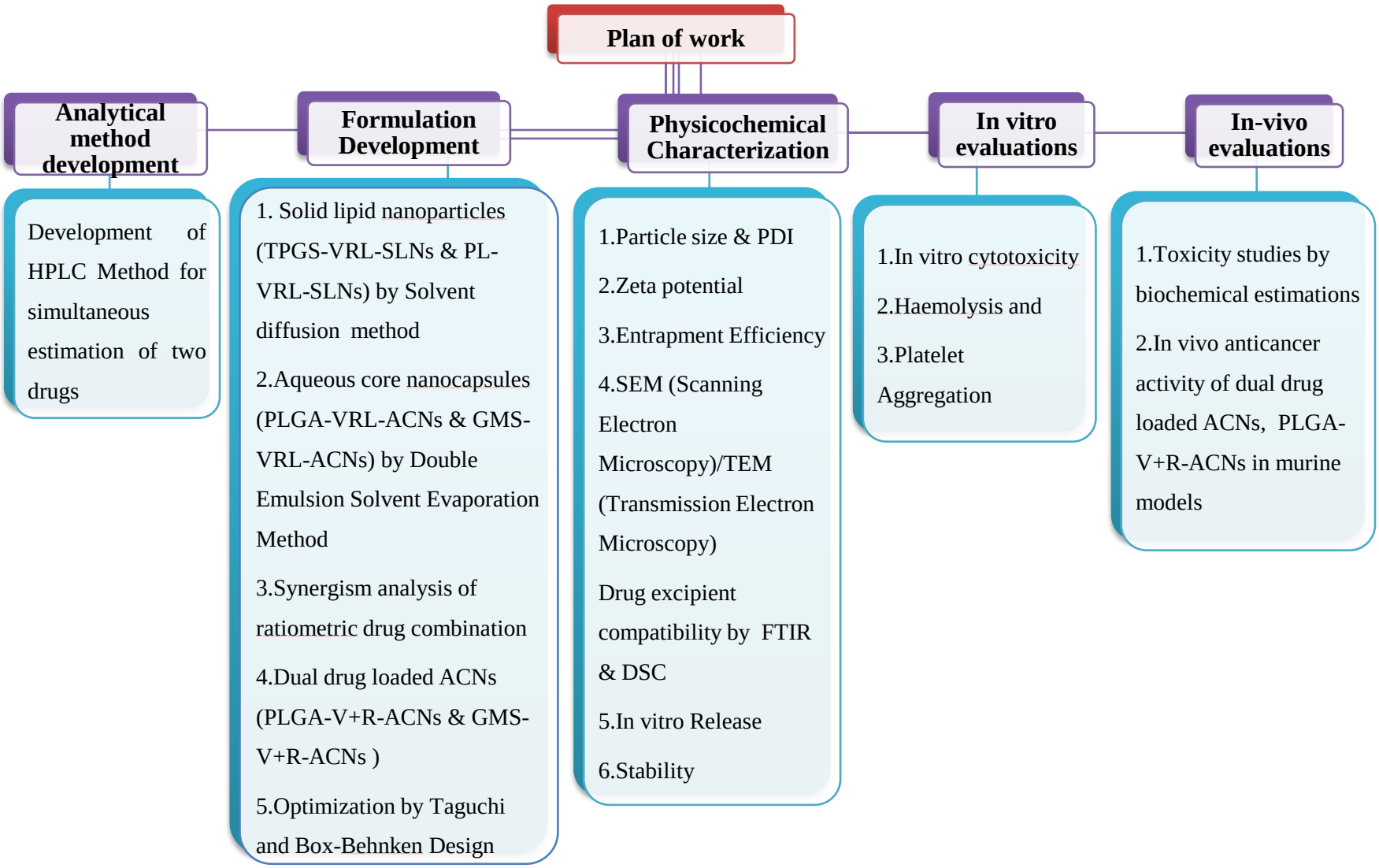
Therefore, there is a demand to develop formulations which can modulate the pharmacokinetics of the combined drugs in such a manner so as to attain the most synergistic combination at the targeted site which becomes more important when the degree of synergy depends on the ratios of combined drugs (Mayer et al., 2006, Tardi et al., 2009).

Novel drug delivery systems like liposomes, polymeric nanoparticles, solid lipid nanoparticles, carbon nanotubes and nanocapsules are already reported to enhance the anticancer efficacy of drugs and can efficiently overcome adverse effects caused by anticancer therapy (Agrawal et al., 2017b, Singh et al., 2016, Vijayakumar et al., 2016a, Vijayakumar et al., 2016b, Vijayakumar et al., 2016c). Combination therapy requires carriers which can encapsulate drugs with different physicochemical properties. Moreover encapsulation of both hydrophilic and lipophilic moieties predisposes wide applications in anticancer therapy which necessitates combination therapies (Cosco et al., 2015, Kothamasu et al., 2012, Vrignaud et al., 2013). In this regard aqueous nanocapsules exhibiting a core shell structures where the core acts as a liquid reservoir and shell a protective membrane(Anton et al., 2009, Kothamasu et al., 2012) can be considered suitable. However, low bioavailability, high plasma protein binding, venous irritation nature and dose dependent side effects demands new strategies to improve therapeutic efficacy and patient incompliance associated with VRL

Considering the problems associated with present VRL therapy, we hypothesize the design and development of nanocarrier based approaches for delivery of VRL alone and in combination with an anti-proliferative antioxidant i.e. RES. Nanocarriers, by virtue of their superior encapsulation and release behaviour in vivo, improve the safety profile of the encapsulated drug. Combination of VRL with RES and their co-encapsulation in nanocarrier

systems may improve enhanced anticancer activity against breast cancer by reducing the dose of VRL in the combination therapy.

8.3 Plan of work - Flowchart



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