Trends in Microbiology

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REVIEWS

Review

The Promise of Long-Acting Antiretroviral Therapies: From Need to Manufacture

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Antiretroviral therapy has transformed human immunodeficiency virus infections from certain death to a manageable chronic disease. Achieving strict adherence to drug regimens that limit toxicities and viral resistance is an achievable goal. Success is defined by halting viral transmission and by continuous viral restriction. A step towards improving treatment outcomes is in long-acting antiretrovirals. While early results remain encouraging there remain opportunities for improvement. These rest, in part, on the required large drug dosing volumes, local injection-site reactions, and frequency of injections. Thus, implantable devices and long-acting parenteral prodrugs have emerged which may provide more effective clinical outcomes. The recent successes in transforming native antiretrovirals into lipophilic and hydrophobic prodrugs stabilized into biocompatible surfactants can positively affect both. Formulating antiretroviral prodrugs demonstrates improvements in cell and tissue targeting, in drug-dosing intervals, and in the administered volumes of nanosuspensions. As such, the newer formulations also hold the potential to suppress viral loads beyond more conventional therapies with the ultimate goal of HIV-1 elimination when combined with other modalities.

Highlights

Long-acting parenteral antiretroviral drugs (antiretrovirals, ARVs) can improve regimen adherence, limit toxicities, and reduce viral resistance.

Prodrug ARV formulations increase the apparent half-life and facilitate drug entry and retention into infectious reservoirs.

Long-acting slow effective release antiretroviral therapies (LASER ART) are hydrophobic lipophilic ARV nanocrystals with a defined 200 to 400 nm size.

Controlled prodrug release and slowed hydrolysis can prolong half-life, improve pharmacokinetic profiles, and facilitate native ARV tissue biodistribution.

LASER ART can lower viral transmission rates, improve treatment outcomes, and facilitate pre-exposure prophylaxis regimens in virus-infected or susceptible individuals.

Scale-up by good laboratory and manufacturing practices facilitates LASER ART translation.

Current Long-Acting (LA) Antiretroviral (ARV) Drug Formulations

Oral administration of antiretroviral (ARV) drugs is the major delivery route for treatment and prevention of HIV-1 infection. While oral delivery focuses on aqueous drug solubility [1], LA hydrophobic ARV formulations affect bioavailability and pharmacokinetics (PK) [2–4]. These can improve regimen adherence and reduce viral drug resistance [5,6]. To this end, in August 2018, ViiV Healthcare announced results from their Phase III Antiretroviral Therapy as Long-acting Suppression (ATLAS) study (clinicaltrials.gov identifier: NCT02951052) where two long-acting drugs, cabotegravir (CAB) and rilpivirine (RPV), were used for the treatment of chronic HIV-1 infection [3,7]. The results of this once-a-month LA injectable paralleled the standard of care of daily oral three-drug regimens[i]. The ATLAS study entered infected adult patients who had previously maintained viral suppression for greater than 6 months on daily oral regimens [comprised of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a third agent]. Rates of viral suppression were equivalent between a continued three-drug oral therapy or after switching to the long-acting, two-drug injectable CAB/RPV regimen. Moreover, the safety and drug-resistance profiles were also consistent with results obtained from both Long-acting Antiretroviral Treatment Enabling LATTE-1,2 regimens [2,8]. Thus, the era of LA ARVs has begun. The questions now emerging are which platform, what drug combination, and what dosing intervals can be achieved for the next-generation LA ARVs. Attention is made to the manufacture, stability, and use of medicines for prevention, treatment, and transmission of viral infections. This review provides a platform for discussion of current and future directives of new ARV treatment regimens. An eye to how each can, and would be, used in future therapeutic regimens designed to combat HIV-1 infections is discussed.

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Chemical Approaches to Facilitate LA ARV Development

With LA ARVs in hand, the question is now: what comes next? One answer is encapsulation of existing ARVs into nanoparticles [5,7,9–14] and the second is chemical ARV modifications to produce hydrophobic prodrug nanocrystals [15–19]. The former requires sequential high-volume injections. The prodrug approach has the advantage of controlled hydrolysis, lipophilicity, and avid mononuclear phagocyte (MP; monocyte and macrophage) depot formation [19,20]. Success in extending the apparent drug half-life from hours to weeks or months was achieved with dolutegravir, cabotegravir, abacavir, and lamivudine (DTG, CAB, ABC, and 3TC) [16–19, 21–23]. Each has the added advantage of achieving sustained native drug levels in blood, lymph nodes, spleen, brain, the genitourinary system, and the gut. Defined chemical modification ensures that each of the prodrugs can be simply and stably manufactured [18,21,22,24]. Notably, improvements in prodrug particle uptake, retention, release, and antiretroviral potency were achieved [16–19,23]. The chemical characterization methods nuclear magnetic resonance, X-ray diffraction, Fourier-transform infrared spectroscopies, and mass spectrometry are the benchmarks for developing modified prodrugs. These methods enable physicochemical characterizations that are compared with water and octanol solubility, acidic and basic pH stability, and prodrug pro-moiety cleavage to yield active drugs. Particle size, zeta potential, stability, and intracellular distribution can predict activity after lyophilization and resuspension. Cell-based retention of drug particles can predict PK profiles.

While CAB and RPV in their native active forms are hydrophobic, chemical modifications afford further improvements in their LA profiles. Historically, the hydrophobic properties of both facilitated their development as aqueous nanosuspensions. CAB LA, a potent integrase strand transfer inhibitor designed as a dissolution-controlled depot ARV formulation, possesses a unique resistance profile with low aqueous solubility [9,10]. When placed in an aqueous 200 mg/ml nanosuspension in polysorbate 20, polyethylene glycol 3350, and mannitol, CAB demonstrates a long half-life [25]. Likewise, RPV LA is stabilized by poloxamer 338 with drug concentrations of 300 mg/ml in aqueous suspensions [4]. Intramuscular administration of both CAB and RPV LA forms depots at the injection site, resulting in sustained drug release and circulation times [26]. RPV LA, which affords a weak barrier to resistance, requires refrigeration and light protection. Both CAB and RPV LAs are manufactured by top-down wet milling techniques, resulting in particle sizes of 200 nm sterilized by gamma irradiation. CAB and RPV LA are administered at 800 and 1200 mg doses, respectively, as 2 ml gluteal injections [27]. Thus, a significant limitation rests in the high dosing volume requirement and resultant injection site reactions. These limitations, together with limited formulation access to ‘putative’ cell and tissue viral reservoirs, open up opportunities for improvements [2,18,28]. The means to further reduce injection volumes while extending dosing intervals are active areas of research.

Implantables

Not mutually exclusive to the use of LA ARVs are implantables. These are placed subcutaneously to provide sustained ARV release [29–31]. Most contain polymeric matrices or rate-limiting semipermeable membranes. One recent entry is a nonerodible silicone system loaded with tenofovir alafenamide (TAF) [31] fabricated from silicone tubing with axial holes coated with a polyvinyl alcohol polymer membrane matrix to control drug release. The device demonstrated a sustained release profile over 40 days in dogs. It is removable and, as such, can protect against adverse reactions. However, surgical insertion and removal of the implant is a noted limitation in resource-limited settings.

Another example is a drug-eluting implant. This was used to provide prolonged release of 4′-ethynyl-2-fluoro-2′-deoxyadenosine (MK-8591) [29], a potent investigational nucleoside reverse transcriptase translocation inhibitor developed by Merck [32]. The device was fabricated from

polylactic acid, polycaprolactone and polyethylene vinyl acetate biodegradable polymers using hot melt extrusion. The implants achieved sustained drug release at therapeutic concentrations for up to 6 months. While the device is promising, drug release itself must be optimized to ensure safety and efficacy prior to its use.

Vaginal rings, well known for their use as birth-control devices, are also being evaluated for extended ARV release [33–38]. Drugs used in rings are stabilized in either polymeric matrices or cores [33,34,36,39]. For example, a silicone ring incorporating dapivirine (DPV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), was recently developed [40,41]. The ASPIRE (A Study to Prevent Infection with a Ring for Extended Use) study, in Phase III testing, investigated the efficacy of a DPV-containing vaginal ring compared with placebo in preventing HIV-1 infection [38]. The study enrolled African women, aged 18–45 years, at risk for HIV-1 infection. A silicone elastomer matrix drug ring was inserted once every 4 weeks in 2629 women. HIV-1 infection occurred in 71 women in the group that received DPV compared with 97 in the placebo group, yielding HIV-1 incidences of 3.3 and 4.5 per 100 person-years, respectively. The incidence of HIV-1 infection in the DPV group was lower by 27% ($P = 0.046$). A higher rate of protection was observed among women over the age of 21 years but not in younger participants. The differences correlated with reduced adherence in younger women. Safety assessment, PK, and acceptability of the DPV vaginal ring [37] confirmed its therapeutic potential. DPV plasma levels were also comparable in all enrolled women.

An alternative to removable implants are biodegradable drug polymer devices. These are being developed with solid ARV polymer gels to deliver dolutegravir (DTG) for up to 9 months [42]. In this report the gel was fabricated from polylactic-*co*-glycolic acid and *N*-methyl-2-pyrrolidone in a solution of drug dissolved in an organic solvent. Upon injection of the DTG-containing solution, the polymer will solidify at the injection site to form the implant. Its potential advantage is that it does not require surgical removal. However, such devices require insertion by medical professionals and processing temperatures that are required during manufacture may result in ARV degradation. There is also the potential for dumping a large amount of drug from the biodegradable polymers. Moreover, the organic solvent used, and the high viscosity of the gel, could cause injection site reactions [43]. As the implant is biodegradable, any need for removal is a challenge especially at longer times after insertion. These limitations could be overcome by refillable devices that do not require repeated surgical insertion or removal. Indeed, vaginal films and gels loaded with ARVs are also being developed [44,45]. These facilitate sustained release of therapeutic drug for a year and are each replaceable.

Oral Formulations

Among all the mentioned strategies, nanosuspensions or solid drug nanoparticles provide an advantage over others in terms of translation to industrial-level production through spray dry manufacture. A single-step emulsion was developed that employs a templated freeze-drying technique to produce nanosuspensions of efavirenz (EFV) [46]. Nanosuspensions of EFV exhibit reduced cytotoxicity and increased drug absorption in Caco-2 cells. *In vivo* analysis of these solid nanoparticles show improved drug PK [higher C_{max}, C_{min}, and area under the curve (AUC)] in rats compared with EFV. EFV nanosuspensions at a 300 mg dose could equal 600 mg EFV regimens [46]. Similar nanosuspensions show improved oral bioavailability for maraviroc [47]. Oral formulations are of particular interest for pediatric patients. The current standard formulation of ritonavir (RTV)-boosted lopinavir (LPV) includes 42% ethanol relative to the drug to solubilize LPV, which has poor aqueous solubility and is undesirable for pediatric patients. Nanosuspensions of LPV produced by emulsion templated freeze-drying provided plasma drug concentrations similar to conventional LPV following oral administration [48]. Improvements for LPV formulations were made by antisolvent precipitations followed by

high-pressure homogenization and step-wise freeze-drying cycles [49]. These provide the potential for improved oral bioavailability over unformulated RTV-boosted LPV and demonstrate the potential elimination of RTV boosting [49].

Further modifications were made by the advanced film-coated gastro-resistant 'Nanoparticle-in-Microparticle Oral Delivery System (NiMDS)' for oral administration of darunavir/RTV (DRV/r) combinations [50]. This system can overcome the limitations of protease inhibitors, including poor water solubility at intestinal pH and greater gastric solubility. NiMDSs are pure nanoparticles of DRV/r encapsulated within film-coated microparticles. DRV and RTV nanoparticles are synthesized by sequential nanoprecipitation/solvent diffusion and evaporation, employing sodium alginate as a stabilizer, and then encapsulated within calcium alginate/chitosan. A series of polymethacrylate copolymers with differential solubilities in the gastrointestinal tract film-coat the particles. The microparticles ensure stability under gastric-like pH. PK analysis in rodents showed that DRV/r-loaded NiMDS increased the oral bioavailability of DRV by 2.3-fold. The approach highlights NiMDSs for improving oral PK [50]. Recently, a novel gastric-resistant oral dosage was developed with an elastomeric core attached to six arms composed of a rigid structural polymer [51]. These rigid arms serve as carriers for the drug-polymer matrices which can achieve constant and sustained plasma DTG, RPV, and CAB concentrations for up to 1 week. However, product complexity and dosing remain a major hurdle for development.

Coformulations

Coformulation of multiple ARVs has drawn growing interest in recent years [14,52–56]. Packaging of multiple ARVs into a single dosage form could potentially improve targeting of different viral replication cycle stages and, as such, minimize viral resistance. In addition, codelivery of long-acting multiple ARVs could improve patient adherence. Most recently, the development of multidrug lipid nanoparticles encapsulating atazanavir (ATV), RTV, and tenofovir (TFV) was reported [56]. These nanoparticles were fabricated by dissolving lipids in chloroform and ethanol followed by drying, rehydration, and homogenization with drug suspensions. While development of multiple-agent-based long-acting formulations could simplify timing of dosing schedules, combinations of compounds within a single lipid nanoparticle introduce challenges. This includes the use of organic solvents during processing, an increased potential for drug–drug interactions, limited lipid stability and reduced ability to independently control drug release. A number of lipid-based drug combinations were developed [53–56]. Investigators evaluated the PK profile of RTV-boosted ATV (ATV/r) and TFV lipid particles in non-human primates following a single subcutaneous injection and compared the PK profile with that of free drug and lipid-stabilized formulations of LPV/r and TFV [56]. After a single subcutaneous administration of the ATV, RTV, and TFV particles, drug concentrations were sustained for 14 days whereas native drugs were detected for only 1–2 days. Lymph node mononuclear cells showed significant levels of all three drugs by week 1 in lipid particle-treated animals. While the platform for combination therapy is an advance, the dosing intervals remain a limitation [56]. Nonetheless, lipid ARV particles do provide sustained release and improved PK drug profiles irrespective of their hydrophobic or hydrophilic properties [54,55].

From Nanoformulated Antiretroviral Therapy (NanoART) to Long-Acting Slow Effective Release Antiretroviral Therapy (LASER ART)

Sequential steps are notable in the development of LA ART. The first was procurement of specific hydrophobic ARVs and then encasing them into particles that were later homogenized to optimize size and shape. The next was attachment of targeting systems to the particle surface that would facilitate depot formation and viral cell targets. Indeed, the potential of targeted nanoART to improve biodistribution and extend the half-life of hydrophobic ARVs from days

to weeks was shown following intramuscular administration in rodents and non-human primates [57,58]. Targeted delivery of ARVs to $CD4^+$ T cells and macrophages has potential in the treatment of HIV-1 infection, as these cells are the primary targets for HIV. This can be achieved by attachment of specific peptides or proteins on the surface of the delivery systems, thereby maximizing binding and interactions between receptors expressed on target cells and delivery systems. Cell-targeted nanomedicines could offer enhanced efficacy, reduced side effects, increased drug stability, and effective subcellular targeting [13,58]. Our own laboratory has developed injectable nanoformulations for different ARVs to facilitate monocyte-macrophage targeting [13,58]. Folic acid (FA) was used as a targeting ligand to deliver drugs to monocyte-macrophages. FA was covalently conjugated onto poloxamers and used to manufacture nanosuspensions of the ARVs by high-pressure homogenization [13,58]. FA-conjugated poloxamer-407 (P407), a biocompatible poloxamer surfactant, was used to form drug nanocrystals containing ATV/r [13]. The FA coated ATV and RTV nanoparticles (referred to as FA nanoATV/r) significantly enhanced drug uptake, retention, and antiretroviral activities. No cellular toxicity was observed. Enhanced retentions of FA-nanoATV/r within recycling macrophage endosomes was observed, which confirmed the stable subcellular drug depot. PK evaluation in mice showed that a single intramuscular injection of FA-nanoATV/r enhanced ATV plasma concentration. Moreover, the ATV concentration in lymph nodes was increased nearly fourfold and in liver and kidneys by up to fivefold [13]. Furthermore, enhanced viral suppression was observed in human peripheral blood lymphocyte-reconstituted HIV-1_{ADA} infected *NOD.Cg-Prkdc(scid)Il2rg(tm1Wjl)/SzJ* mice treated with FA-nanoATV/r compared with untargeted nanoATV/r [58]. The development of anti-CD4 modified liposomes loaded with both nevirapine and saquinavir was described [59]. The prepared liposomes were made by thin-film hydration and covalently linked to a CD4 antibody demonstrating improved cellular drug uptake and antiretroviral activity compared with native drugs. However, regardless of the targeting efficiencies, the complexities of manufacture limited their development.

Other limitations of nanoART include rapid ARV metabolism and limited biodistribution [12,13,57,58,60–66]. These limitations can be overcome through chemical modifications of native ARVs to generate lipophilic and hydrophobic prodrugs into LASER ART nanocrystals. The true advantages of LASER ART are in permitting rapid drug penetration across physiological barriers, slow drug dissolution, poor aqueous solubility, enhanced bioavailability, and reduced systemic toxicities [17,18,24,67]. LASER ART is defined as hydrophobic prodrug crystals stabilized by lipids or surfactants enabling sustained release of therapeutic drug concentrations in plasma and transfer across anatomical viral reservoirs [15]. The inactive LASER ART prodrug is metabolized into the native active drug by enzymatic or chemical hydrolysis of the 'masking group'. Prodrugs are a major component of LASER ART and enable loading of >80% with improved membrane permeability [68–70].

Ester prodrugs of lamivudine (3TC), abacavir (ABC), DTG, and CAB were synthesized through myristoylation to generate M3TC, MABC, MDTG, and MCAB. A DTG prodrug nanoformulation (NMDTG) was prepared by myristoyl ester modification [17]. NMDTG particles showed enhancements in macrophage drug uptake, prolonged retention, and drug efficacy for up to 1 month. PK tests in *Balb/cJ* mice showed blood and tissue DTG levels at, or above, the protein-adjusted 90% inhibitory concentration (PA-IC_{90}) for up to 56 days after a single 45 mg/kg intramuscular injection. NMDTG injection (118 mg/ml; 25.5 mg/kg DTG equivalents) into rhesus macaques resulted in plasma DTG levels of up to 86 and 28 ng/ml on days 35 and 91, respectively [21], without adverse events [17,21]. Similarly, intramuscular administration of nanoformulated CAB prodrug at 45 mg/kg CAB equivalents provided plasma drug levels four times above the PA-IC_{90} (660 ng/ml) for 56 days in rhesus macaques and above the 1x PA-IC_{90} (166 ng/ml) for 13 weeks in mice [18,22].

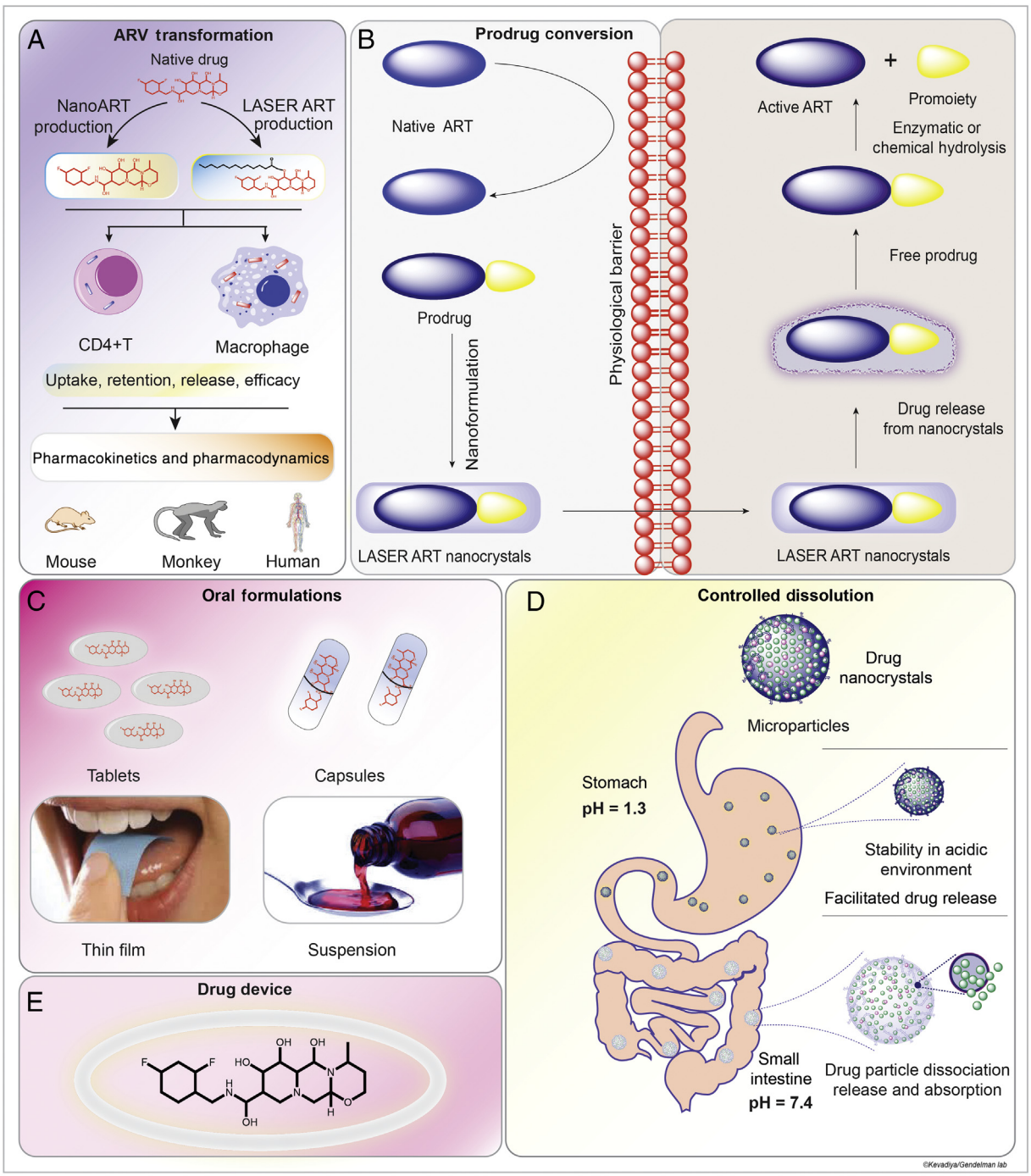

Figure 1. Generating Long-Acting Slow Effective Release Antiretroviral Therapy (LASER ART).

For a Figure360 author presentation of Figure 1, see the figure legend at https://doi.org/10.1016/j.tim.2019.02.009.

The plates serve to review each of the delivery schemes either developed now or being actively researched for oral, device-linked, or parenteral administrations. (A) Schematic illustration of nanoformulated native drug (nanoART) or long-acting slow effective release ART (LASER ART) as defined by hydrophobic lipophilic

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Novel formulations of ABC by PROdrug and nucleoTIDE (ProTide) technology (NM3ABC) were produced [19]. Three (NM1ABC, NM2ABC, NM3ABC) were made. Among these, M3ABC, showed the highest drug encasement efficiency, produced the highest intracellular carbovir-triphosphate (CBV-TP) levels, and formed a long-lived cell-based depot. CBV-TP was observed over time in lymphocytes [19]. While LASER ART can extend drug interval dosing, it may supplant other available options considering any limitations in adverse events, injection volumes, ease of distribution, and PK parameters (Figure 1). Altogether, these data demonstrate that thoughtfully engineered prodrugs can improve PK and ARV biodistributions over-encapsulation of native drug.

Pharmacokinetic Modeling

Models are sometimes used to predict pharmacokinetics (PK) after dosing in animals and humans. These models define timed correlations between relevant tissue and plasma drug concentrations. The PK can be predicted by incorporating physicochemical properties and *in vivo* behavior of drug molecules in the design of such mathematical representations [71]. Physiologically based pharmacokinetic (PBPK) models are based on the anatomical individual tissue compartments and drug movement and are used as tools for predicting PK and biodistribution of drug molecules [72,73]. The limitations in PBPK reside in often complex formulation and prodrug design, variant *in vivo* drug biodistribution, metabolism, and transport [74]. Nanoparticle size, shape, and surface characteristics all affect nanoparticle distribution. While some nanoparticles are designed for uptake by the reticuloendothelial system, others are carried through the lymphatic system [75,76]. The first nanoparticle experimentally-based PBPK model was designed for doxorubicin-loaded liposomes [77]. Since then a number of nanomaterials have been used to fit PBPK models to predict biodistribution [78]. Recently such models were used to simulate the PK of long-acting antiretroviral drug formulations validated against clinical data for better prediction of drug dose optimization, including release rates from the intramuscular injection depot [79]. PK predications and dose optimizations for the antiretroviral drug EFV were developed with simulation and computational modeling [80]. Furthermore, the same approach was used to predict the PK of CAB LA and RPV LA formulations in adults and children by considering absorption, distribution, metabolism, and drug excretion [81]. Despite the growing interest in modeling to predict nanoformulated ARV PK, there are challenges that restrict the application of PBPK modeling and simulation for human drug PK prediction. First, PBPK models require more clinical and *in vivo* experimental data when compared with traditional animal PK models [82]. Second, clinical variables abound, and too many experimental parameters would be required to develop an ideal PBPK model, including predictability problems from *in vivo* animal to human clinical trial studies and data collection variability from person to person [83–85]. Third, routes of administration and absorption rates for nanoformulations, including transdermal, oral, intravenous, intramuscular, and vaginal administration, are each unique in animals and humans and introduce ambiguity into PK parameters [86]. Fourth, a lack of suitable data sets provides poor knowledge of physiologic conditions, biochemical changes, and tissue-specific enzymes

prodrugs. Nanocrystals are developed containing antiretrovirals (ARVs) (for example, dolutegravir). Surfactant-stabilized nanocrystals are prepared by high-pressure homogenization or wet milling. Cell-based assays are used to screen drug potency, cytotoxicity, uptake, retention, release, and efficacy. The top-performing formulations are then moved forward for safety, pharmacokinetic and pharmacodynamic assessments. (B) The prodrug concept and LASER ART nanocrystal formation, particle uptake, intracellular prodrug release, and slow hydrolysis to extend the apparent half-life of the drug. The ARVs are modified to improve drug potency, enhance cell membrane permeability, and facilitate encapsulation into LASER ART nanocrystals that are rapidly taken up by cells and distributed into lymphoid tissues. (C) Examples of extended-release oral ARV formulations in preclinical development include capsules, tablets, thin films, and suspensions. The ARVs are embedded in a matrix system that controls drug release. (D) pH-sensitive microparticles and devices are being leveraged to control release of ART after oral administration. (E) Subcutaneously implantable devices, vaginal rings, films, and gels loaded with ARVs are at various stages of preclinical development to provide sustained release of ART for HIV-1 treatment and prevention.

that may be seen in some patients. Moreover, variation in the number and type of drug transporters, type and species specificity of cell receptors, drug permeability, and enzyme activities can result in inaccurate predictions [84,87]. Lastly, the PKs of highly hydrophobic drugs with different sizes and shapes of nanoformulations are difficult to predict due to high drug-protein binding and difficulty in mimicking the exact plasma environment [88]. Finally, PBPK models may require future modifications as more diverse chemical and physicochemical alterations are made in advanced formulations [89]. These could be obviated through the development of ARV theranostics.

Theranostics: ARV Particle Detection and Therapeutic Efficacy

Theranostics is an emerging discipline that uses agents that allow simultaneous measures of diagnoses with therapeutic deliverables. Biodegradable and biocompatible nanoparticles hold an important role in the theranostics field and allow combination of diagnostic and therapeutic properties with desired cell and tissue targeting for a number of diseases (Figure 2). Over the last decade, advances in surface chemistry of theranostic nanoparticles has enabled the development of more specific individualized therapies for various diseases. Theranostic particles can be made by encapsulating a therapeutic drug into imaging nanoparticles such as iron oxide nanoparticles and gold nanoparticles or by tagging imaging probes, including fluorescence dyes and radioisotopes, onto the therapeutic nanoparticles. Encapsulation of imaging and therapeutic agents together in a biocompatible nanocarrier is also practical. Moreover, the use of intrinsic imaging and therapeutic nanoparticles can predict drug efficacy. In general, theranostic nanoparticles provide combinations of organic and inorganic phase encasements, allowing drug delivery by organic nanoparticles and bioimaging made possible by added inorganic components [90,91].

The realization of theranostic particle use rests in its abilities to accurately measure drug distribution and predict PK profiles. In conjunction with radiolabel and fluorescence probe, drug-loaded nanoparticles can allow PK and real-time drug distribution analyses. Biodistribution of drug-loaded particles depends upon drug properties, including lipophilicity, the extent of loading, encapsulation, and hydrophobicity, as well as particle size, shape, surface chemistry, and the inherent cell and tissue physicochemical properties [15,92]. Moreover, theranostic particles can facilitate drug concentration measures in reservoir sites to assess drug depot formation. Superior spatial resolution afforded by noninvasive positron emission tomography, computed tomography, magnetic resonance imaging, and single-proton emission computed tomography imaging enables accurate real-time biodistribution studies of theranostic particles with high resolution and sensitivity. Recently, using organic–inorganic hybrids, we developed stable FA-decorated europium (Eu^{3+})-doped cobalt ferrite (FA-EuCF) nanoparticles encapsulating DTG. The decorated particles can be readily taken up by macrophages to establish a reticuloendothelial system drug depot and have similar biodistribution in Sprague-Dawley rats and rhesus macaques [92]. Other multimodal ^{111}indium (^{111}In) radiolabeled EuCF-RPV theranostic particles have also provided a reliable estimate of drug biodistribution [93]. Thus, multimodal theranostic nanoparticles are a promising tool in early disease diagnosis. While theranostic nanoparticles are thought to be limited to the study of drugs with a long circulation half-life, porphysome nanoparticles allow more efficient targeting with subsequent activation of photodynamic activity to eliminate cancers or infected cells [94]. Studies performed with superparamagnetic iron oxide nanoparticles, functionalized with gelatin-oleic acid, show enhanced PK and targeted biodistribution of the nanoparticles [95].

Good Laboratory Practices (GLPs) and Good Manufacturing Practices (GMPs)

The overall objective in producing LA antiretrovirals is to reduce the stigma of daily drug administration and improve therapeutic compliance. Prodrugs are made to extend the

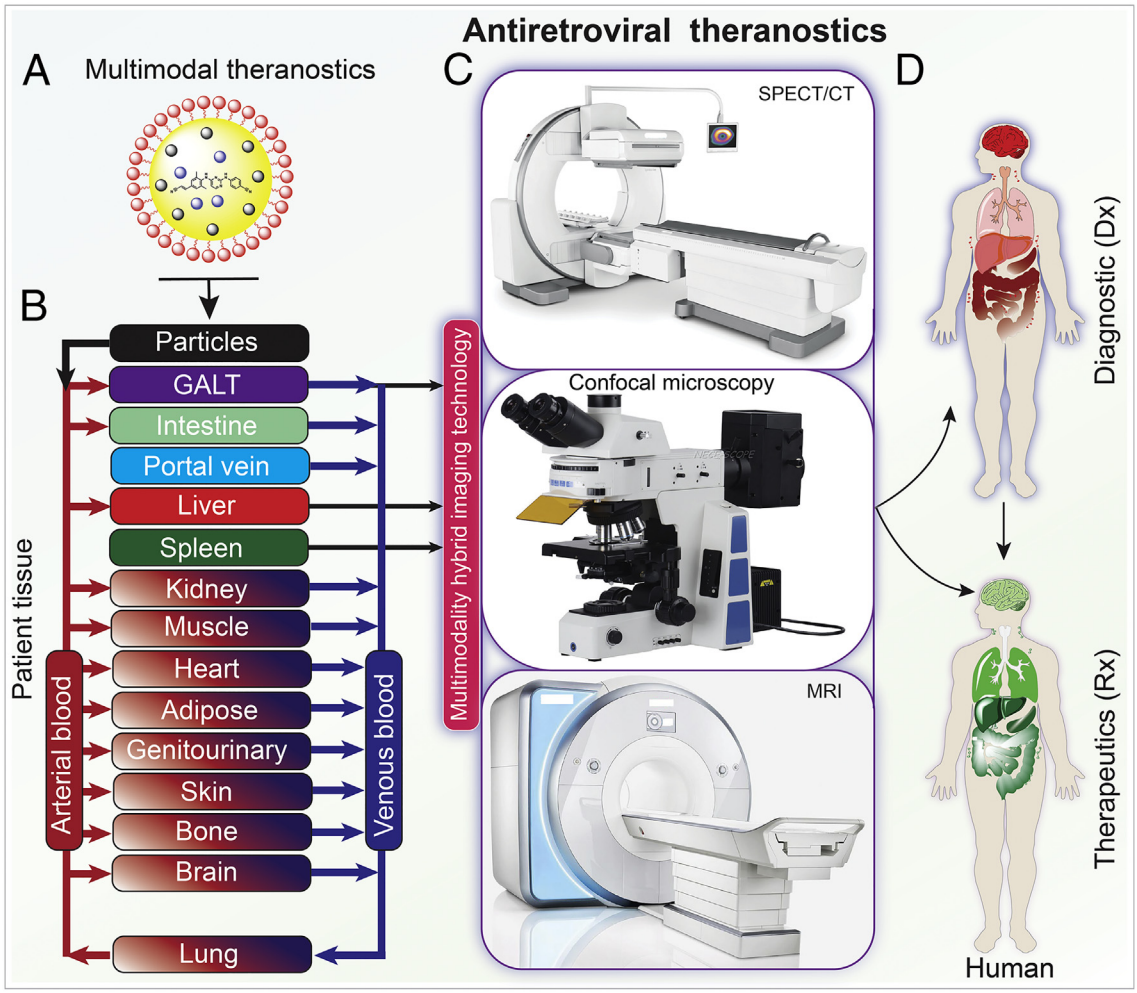

Figure 2. Theranostic Multimodal Nanoparticles Predict Drug Delivery to Infectious Tissue Sites. (A) Theranostic particles are made with multifunctional capabilities for delivery to virus-infected, inflamed tissues, or after organ injuries. The schematic illustration denotes the surface-targeting and internal drug, nucleic acid and/or imaging payloads that form the backbone of the nanoparticle. (B) Following parenteral injection, biodistribution is seen in each of the listed tissues with preference in HIV-1-infected lymphoid organs, brain, and the reticuloendothelial system, but not excluding the kidney, bone, muscle, heart, and skin amongst others. (C) The multifunctional particles containing metal, isotope, and/or fluorescence encasements can be used to assess drug biodistribution through bioimaging. Modalities include, but are not limited to, single-photon-emission computed tomography-computerized tomography (SPECT/CT), confocal microscopy, and magnetic resonance imaging (MRI) amongst others to track particle biodistributions. (D) Diagnostic and therapeutic payloads contained within the theranostic particles reach their cell and tissue destinations at levels reflecting the extent of disease, infection, inflammation, or degeneration (in red) then ameliorate the disease process or restrict/eliminate an infection. The bioimaging of the particles can then define time, place, and drug levels in real time, enabling delivery of the therapeutics that combat disease events. Altogether, these methods provide real-time particle tracking, biodistribution, and treatment of disease (in green). Abbreviations: GALT, gut-associated lymphoid tissue.

apparent half-life of the drug and facilitate their entry into tissue sites of active viral growth. Hydrophobic prodrugs are readily nanoformulated and can be made to cleave slowly to their active moieties [20,68,96]. However, their translational potential [97,98] could be limited by inherent complexities and scale-ups [99–101]. Thus, creation of specific GLP and GMP facilities that provide specializations for formulation manufacture are essential. If safely and efficiently produced, LA ARV nanoparticles can serve as drug storage depots in macrophage

subcellular compartments [102–105]. In this manner, the particle's cell retention would servie to contribute to its LA antiretroviral activities [103,105,106]. Synthesis would produce nanomedicines that could affect trafficking to acidic lysosomal compartments where low pH could be harnessed to degrade/hydrolyze the nanoformulations where active drug would be released [107]. Attention to each of these must be part of any proper evaluation of GMP manufacturing to ensure proper development and efficient clinical translation. There are additional and inherent challenges in translating nanomedicines into clinical entities [108,109]. Indeed, the structural and chemical complexity of nanomedicines can affect scale-up production, batch-to-batch reproducibility, formulation stability, and sterilization [101,110,111]. With these hurdles in mind, protocols for scale-up and formulation reproducibility were developed for LASER ART then tested in large animals for long-acting activity and toxicities [21,22,24]. GLP protocols ensured uniformity, consistency, reliability, reproducibility, quality, and integrity of pharmaceutical products[ii]. GMP scale-up production and product quality assessments follow, ensuring that product translation is performed from bench to bedside in a multistep, simple, and interactive manner[iii] (Figure 3).

Small-animal model systems provide screening PK and pharmacodynamic (PD) assessments. In these systems optimizing product delivery, drug tissue targeting, metabolism, disease outcomes, and potential toxicities can be addressed. For LASER ART immune-deficient mice reconstituted with human immune cells allow assessments of pre-exposure prophylaxis and antiviral responses [112–114]. However, large animals, that include rhesus macaques, need to be employed for confirmatory PK and PD tests [115–117] as species differences commonly occur in drug metabolism [118,119].

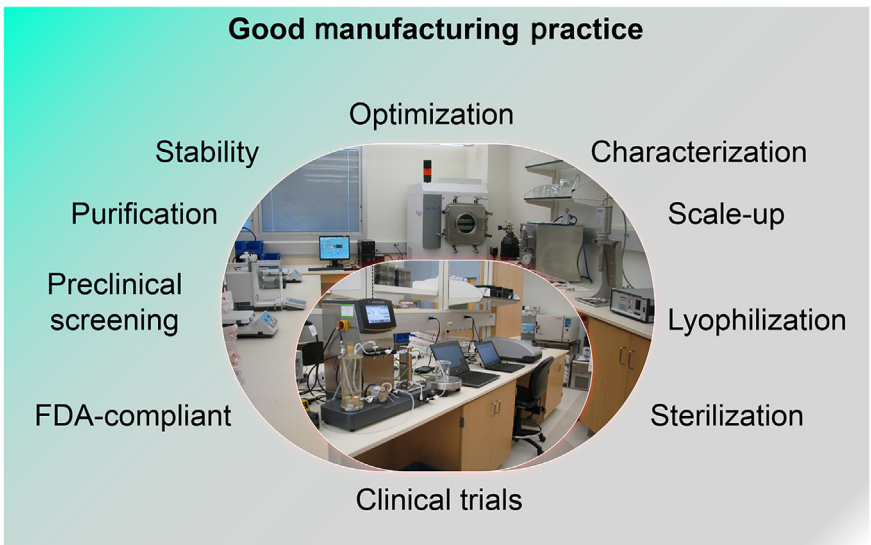

Figure 3. Current Good Manufacturing Practices (cGMPs). The Nebraska Nanomedicine Production Plant (NNPP) reflects standards set forth by the USA Food and Drug Administration (FDA). The facility is compliant with GMP guidelines. Formulation development involves standard protocols for preclinical screening, particle purification, a range of stability tests, optimization of nanoparticle production, and in-depth characterizations. These are first conducted using good laboratory practices (GLP) and defined cross-observational protocols. GMP follows strict USA FDA pharmaceutical quality manufacturing standards for product scale-up and sterilization. These lead to the production of high-quality products for human use that include high-impact lyophilized pharmaceuticals.

Finally, to prepare nanoformulations for preclinical safety and Phase I clinical studies, the nanoformulation production, composition, and stability must be optimized by GLP and GMP protocols and guidelines (Figure 3[iii]). Producing sufficient quantities of nanomedicines for Phase I clinical studies requires GMP-validated and strict quality-assurance measures. Immune and metabolic testing for safety, and the use of electron-beam or gamma irradiation for sterility help to ensure that the final nanomedicine product can meet the requirements of an investigational new drug application with the completion of all USA FDA requirements. To achieve this goal for LA ARVs, we created our own Nebraska Nanomedicine Production Plant GLP and GMP facility. Like others, it enables optimized scale-up of developed formulations and manufacturing of product for clinical testing.

Concluding Remarks and Future Perspectives

The realization of long-acting prodrug ARV formulations from the laboratory to clinical use is as of now a challenge (see Outstanding Questions). To truly produce a superior product the long-

Outstanding Questions

Can improved ARV biodistribution into infectious reservoirs affect treatment outcomes?

Are there longer-term toxicities from sustained release LA ARVs?

Can improved pharmacokinetic and pharmacodynamic profiles seen by LASER ART facilitate viral elimination by immune, clearance, or excision schemes?

If toxicities emerge from long-acting ARVs, can the drug be removed?

Can pre-exposure prophylaxis regimens, at 6-month, or longer, dosing frequencies lead to viral eradication?

Will scalable LASER ART regimens be embraced by patients and care-givers?

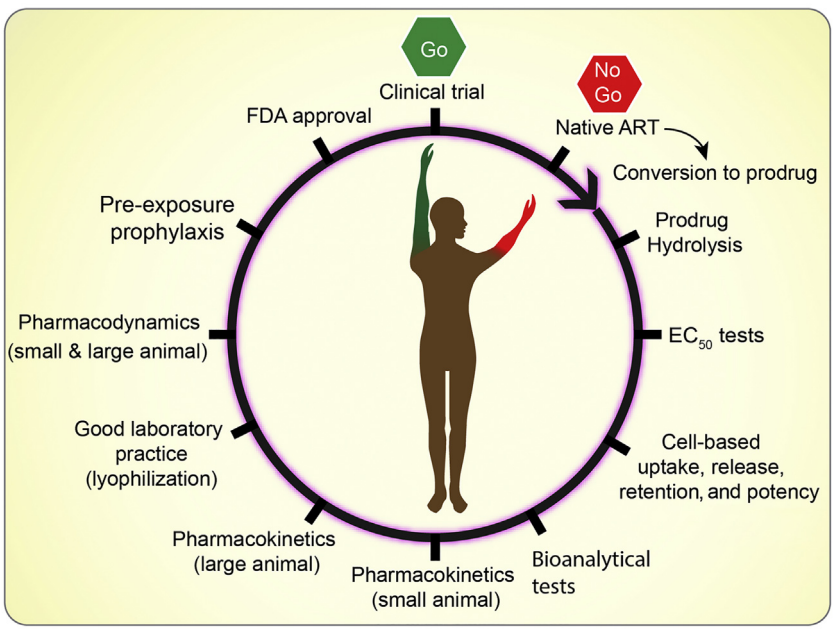

Figure 4. Developmental Testing of Prodrug Formulations. Design for chemical modifications of a native antiretroviral (ARV) is forged into go no go 'clock' criteria. Cabotegravir (CAB) is given as an example. CAB was first transformed into a lipophilic hydrophobic prodrug. The transformation was screened by prodrug hydrolysis with plasma esterases during timed incubations. Assessment of half-maximal effective concentration, or EC_{50} reflective of drug potency, was made for ARV responses halfway between baseline and maximum based exposures. The native drug and prodrug, with or without nanoencasements, were tested for uptake, release, retention, and ARV activities in primary human $CD4^+$ T cells and macrophages. Bioanalytical testing includes drug and prodrug stability over time, temperature, and pH. Measurements of drug and prodrug levels inside and released from cells are routinely performed. In the subsequent evaluation, nanoformulated prodrug was subjected to pharmacokinetic testing and tissue biodistribution in rodents then followed by parallel assessments in rhesus macaques. This then proceeded to full good laboratory practice (GLP) guideline testing for product use and is assessed by a complete drug-toxicology profile evaluation, as are the preclinical pharmacodynamic testing evaluations for pre-exposure prophylaxis. Ongoing works include serial titered viral exposure through vaginal or rectal routes. Investigational new drug enabling, FDA approval, and good manufacturing practice (GMP) production will precede the first in human testing (Phase I clinical trial). Abbreviation: ART, antiretroviral therapy

acting capabilities and the biological properties of the nanomedicines need to be realized. The formulations must include an ability to cross cell membrane barriers and release drug cargo slowly at infectious sites. The potential viability of active targeting through engagement of cell receptors needs also to be determined in relevant cell and animal models. Active prodrug release from the nanoformulation in subcellular compartments and prodrug hydrolysis need to be determined in relation to any elicited changes to the cellular environment. For example, crystalline ARVs are taken up by macrophages by endocytosis and stored in endosomal compartments. Drug retention in endosomal compartments parallels the assembly of HIV virions and contributes to its long-acting antiviral activity. Other nanomedicines are trafficked to acidic lysosomal compartments where low pH degrades the nanocrystal and releases the active drug. Furthermore, potential toxicities can also be determined in these cell-based systems. Cell-specific uptake and activity and effectiveness against different strains must be screened as well. The key role of cell reservoirs and their interactions with ARV nanocrystals must be uncovered if such novel treatments can be realized (Figure 4).

Resources

[i]www.viivhealthcare.com/en-gb/media/press-releases/2018/viiv-healthcare-reports-positive-48-week-results-for-first-pivotal-phase-iii-study-for-novel-long-acting-injectable-hiv-treatment-regimen/

[ii]www.gmppublications.com/Part58GLP.htm

[iii]www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210

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