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Nanotechnology-Based Targeted Drug Delivery: An Emerging Tool to Overcome Tuberculosis

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The appearance and rapid spread of drug resistant strains of tuberculosis (TB), one of the deadliest infectious diseases, pose a serious threat to public health and increase the need for shorter, less toxic, and more effective therapies. Developing new drugs is difficult and often associated with side effects, so nanotechnology has emerged as a tool to improve current treatments and to rescue drugs having elevated toxicity or poor solubility. Due to their size and surface chemistry, antimicrobial-loaded nanocarriers are avidly taken up by macrophages, the main cells hosting *Mycobacterium tuberculosis*. Macrophages are continuously recruited to infected areas, they can transport drugs with them, making passive targeting a good strategy for TB treatment. Active targeting (decorating surface of nanocarriers with ligands specific to receptors displayed by macrophages) further increases local drug concentration, and thus treatment efficacy. Although in in vivo studies, nanocarriers are often administered intravenously in order to avoid inaccurate dosage in animals, translation to humans requires more convenient routes like pulmonary or oral administration. This report highlights the importance and progress of pulmonary administration, passive and active targeting strategies toward bacteria reservoirs to overcome the challenges in TB treatment.

1. Introduction

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB), has plagued humankind since antiquity. Despite its long history, TB is currently one of the top 10 causes of death worldwide and the deadliest infectious disease, ranking above human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS).[1] In 2018, an estimated 10 million people developed TB and 1.5 million died from the disease (including 251 000 deaths among HIV-positive), which is equivalent to a staggering 4000 deaths per day.[1] According to the last report from the World Health Organization (WHO), 23% of the global population is latently infected with *Mtb*, meaning that they are asymptomatic and cannot transmit the infection.[1] However, they have a 5–15% lifetime risk of developing active TB disease, being this rate higher in individuals immunocompromised by HIV coinfection, diabetes, and undernutrition.[2] For this reason, the incidence is much higher in areas where many of these conditions coincide, such as South-East Asia and Africa (accounting for 68% of new cases).[1]

The current frontline treatment for drug-susceptible TB includes a 2-month initiation phase of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (ETB), followed by a 4-month continuation phase of INH plus RIF.[3,4] Treatment success rates of at least 85% for cases of drug-susceptible TB are regularly reported to WHO by its 194 Member States.[1] The main reason for prescribing a combination of anti-TB drugs with

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different modes of action is to reduce the emergence of drug-resistant strains. Yet, irregular drug supply, noncompliance to the treatment, inappropriate drug regimens, and lack of supervision have led to the emergence of resistant strains.[5]

Those *Mtb* strains resistant to the cornerstone drugs RIF and INH are named multidrug-resistant (MDR), whereas the term extensively drug-resistant (XDR) refers to MDR *Mtb* strains with additional resistance to a fluoroquinolone (such as levofloxacin or moxifloxacin (MXF)) and a second-line injectable drug (amikacin (AMK), capreomycin, or kanamycin). Moreover, in the last years, several cases of strains resistant to all first- and second-line drugs used to treat TB have been reported.[6]

During 2018, about 484 000 cases of TB were caused by strains resistant to RIF (RR-TB), of which 78% were MDR-TB. The most affected countries by MDR-TB were India, China, and the Russian Federation, being responsible for half of worldwide cases. Although in the European Region the TB incidence rate fell 15% between 2015 and 2018, the proportion of RR-/MDR-TB cases there (30%) is higher than that in all other regions (3.1–5.4%).[1,7] Regarding XDR-TB, an estimated 6.2% of MDR-TB worldwide cases were XDR-TB in 2018.[1] The current therapies for MDR-TB and XDR-TB are longer (up to 18–20 months) and require the use of second-line drugs, which are more expensive and have more toxic side effects than the first-line drugs. In addition, the use of injectable drugs complicates even more the adherence to the treatments. Hence, the treatment success rate reported in 2018 was 56% for RR-/MDR-TB and 39% for XDR-TB, making drug-resistant TB a threat to global public health.[1,8] Moreover, TB treatment in patients with HIV is further complicated by significant drug–drug interactions between anti-TB drugs and antiretroviral therapies.[9]

Bedaquiline (BDQ, 2012), delamanid (2014), and pretomanid (2019) are the only three anti-TB drugs with novel mechanisms of action approved for the treatment of MDR-/XDR-TB in the last 40 years. There are several regimens for MDR-/XDR-TB under clinical evaluation which include them, such as the NiX-TB trial. This all-oral regimen, recently approved by the Food and Drug Administration (FDA), consists of a combination of BDQ, pretomanid, and linezolid during 6 months for the treatment of XDR-TB and some MDR-TB cases and led to a favorable outcome, however associated toxic effects were observed. The latest WHO guidelines on drug-resistant TB treatment suggest that most RR-/MDR-TB patients can be treated with fully oral regimens, using injectable agents (specifically AMK) only if other options are not possible.[1,8,10,11]

Based on these, there is an increasing need for more effective therapies with less toxicity and shortened treatment duration. Nanotechnology offers several advantages to overcome the limitations of current TB therapy.[12] With the aid of this versatile approach, a sustained and targeted drug delivery can be achieved with the promise of an efficient pulmonary administration to further enhance treatment success.[13] The present progress report provides an overview of the potential use of nanotechnology-based drug delivery toward overcoming the challenges in TB treatment with more emphasis on pulmonary delivery. This report highlights the importance and advances of both passive and active targeting of nanocarriers toward bacteria reservoirs in order to eradicate TB. Finally, this report gives an outlook of the progress of these systems in clinical translation.

2. TB Pathogenesis at a Glance, Know the Enemy

Mtb is an obligate intracellular pathogen, whose unique reservoirs are humans. *Mtb* infection initiates when the bacilli reach the lungs by inhalation of aerosolized droplets expelled from individuals with active pulmonary TB (e.g., by coughing or sneezing). Once in the alveolar space, bacteria are internalized via phagocytosis by alveolar macrophages (AMs), as the frontline cells of the innate immune response. The pattern-recognition receptors (PRRs) present on host cells recognize pathogen-associated molecular patterns (PAMPs) found on the surface of the bacilli, inducing the secretion of cytokines and chemokines that leads to the recruitment of additional phagocytic cells (neutrophils, interstitial macrophages, and dendritic cells) into the site of infection.[14,15] Following phagocytosis, in ≈20–25% of cases, macrophages can fully contain the infection and completely eliminate the pathogen,[16] however, in most cases, *Mtb* manages to escape eradication by host cells.[17] *Mtb* resides in the phagosome and through the activity of its ESX-1 secretion system releases proteins and other molecules to the phagosome, causing the disruption of the phagosomal membrane and subsequently, the release of mycobacterial components into the macrophage cytosol. As a consequence, *Mtb* manages to arrest phagosome maturation by preventing lysosome fusion; then, *Mtb* transforms the highly hostile conditions of the phagosome into a milder environment where it can survive and is able to replicate inside the macrophage.[18,19] Meanwhile, infected cells migrate to pulmonary lymph nodes, where they process and present antigens for T cell priming (both CD4+ and CD8+). After 15–18 days of *Mtb* infection, the adaptive immune response produces primed T cells, which migrate to the site of infection guided by the chemokines produced by infected cells.[20] As a result, the pathogen can 1) be eliminated by host immune response, 2) progress to active disease (mainly in immunocompromised hosts), or 3) persist in structures called granulomas – the pathological hallmark of tuberculosis.[18] A granuloma is a well-organized aggregate of immune cells that arises in response to a persistent stimulus. Granulomas are organized in spherical structures with infected macrophages in the middle, surrounded by neutrophils, dendritic cells, natural killer cells, B and T lymphocytes, fibroblasts, and other matrix components.[21] Macrophages within the granulomas can differentiate into other cell types, such as epithelioid cells, foamy cells, and multinucleated giant cells, formed after fusion of multiple macrophages.[22,23]

The main function of the granuloma is to localize and confine the infection while concentrating the immune response to a certain area. In these structures, the bacilli are exposed to a variety of stressful conditions, such as hypoxia, acidic pH, low iron availability, and nutrient deprivation.[24] In order to overcome these challenging host immune responses, the bacteria have evolved mechanisms to maintain viability with limited or no replication. This state (called dormancy) is characterized by low metabolic activity and a change in the composition and spatial architecture of the cell wall[25] and consequently, bacteria show phenotypic drug tolerance to antibiotics targeting functions required for growth.[26] Therefore, granulomas establish a dynamic balance between host defenses and bacterial dormancy. If the immune system controls the infection in granulomas, then the

individual has latent TB infection (LTBI). LTBI is a controlled asymptomatic and noncontagious infection that is established in 65–75% of cases[1,27] and this stage can last for years or even for life. During LTBI, solid granulomas prevail, which are typically encircled by a fibrotic wall that separates it from the surrounding tissue.[17] However, due to a dysregulation in the immune response, dormant bacteria can reactivate leading to necrosis of infected macrophages. This causes the formation of a necrotic zone in the center of granulomas (known as caseum because of its milky appearance), which contains extracellular *Mtb* released by necrotized foamy macrophages. Caseous necrosis – a hallmark of pulmonary TB pathology – is associated with reduced vascularization of the lesion (hypoxia) and high lipid content, as a result from the death of foamy macrophages.[17,28] Further loss of granuloma integrity allows the entry of vascular oxygen and nutrients, which promotes the growth of the bacteria. Finally, the collapse of the granuloma releases the bacteria to blood capillaries and the alveolar space and disseminates to other parts of the lung and also to other organs (hence producing extrapulmonary tuberculosis), in order to fully develop an active disease (meaning that they present clinical symptoms) and also to be transmitted to other individuals.[1,17–19,29]

2.1. Main Host Cells

Being *Mtb* an obligate intracellular pathogen, the range of different type cells that it can infect is highly diverse, including both from myeloid and lymphoid origin. Upon entry into the lungs, *Mtb* is engulfed by AMs, which are the dominant cell type that the pathogen infects; other cell types that can be infected by *Mtb* are interstitial macrophages and dendritic cells. In all these cases, infection is mediated through recognition by PRRs expressed by host cells, as described above. Other cell types that can host *Mtb* intracellular replication include foamy macrophages, epithelioid macrophages, neutrophils, lymphocytes, and myeloid-derived suppressor cells.[18,19]

It has been described that *Mtb* can also infect human and mice mesenchymal stem cells (MSCs), a type of multipotent cells that can differentiate into multiple cell phenotypes. After MSCs phagocyte *Mtb* mediated by scavenger receptor-A (SR-A), these cells activate autophagy and nitric oxide production in order to kill replicating *Mtb*; however, dormant *Mtb* cannot be eliminated, so when MSCs migrate to bone marrow, *Mtb* will reach a new niche to persist. In fact, in patients that have completed successfully an anti-TB treatment, *Mtb* still can be found in CD27+ bone marrow MSCs.[30,31]

2.2. Main Challenges for TB Treatment

Altogether, TB infection is a dynamic process in which different pathological stages of granulomas coexist (solid, caseous, and cavitary granuloma), so the bacilli must adapt to a variety of microenvironments to survive. Eradication of dormant bacteria is challenging due to their tolerance to TB drugs and the suboptimal drug concentrations reached in the caseum of necrotic granulomas. Consequently, nonreplicating bacteria may serve as reservoirs for TB reactivation and emergence of drug resistance. In addition, a situation similar to LTBI may occur after a drug-based treatment of active disease, when the bacteria are not fully eliminated and those few that are still present in the body can be maintained in a latent condition that may result in relapse after a period of time.[1,27] These factors also help to explain the long duration of TB treatments required to cure active TB. Therefore, to successfully eradicate the infection, it has high priority to reach the bacteria in its host cells inside granulomas.

3. Nanotechnology-Based Drug Delivery Systems

Using nanocarriers in drug delivery is an emerging strategy in the combat against various diseases. The main advantages of nanocarrier systems over free drugs are enhanced bioavailability, protection of the entrapped drug from inactivation, sustained and controlled drug release, and the possibility of reducing the administered doses, and thus the related side effects and administration frequency. To reach the reservoirs of *Mtb*, a variety of nanocarriers have been developed, including polymeric nanoparticles, nanocapsules, micelles, dendrimers, nanogels, liposomes, solid lipid nanoparticles, inorganic nanocarriers, etc. Therapeutic agents can be incorporated into nanocarriers either by physical encapsulation, adsorption, or chemical conjugation. Importantly, using nanocarriers, it is possible to target host cells either by passive accumulation or by active targeting.[12,32]

3.1. Administration Routes of Nanocarriers for Treating TB

The different routes for nanocarrier administration have different limitations depending on the biological environment and barriers that nanocarriers face with. In TB treatment, oral, intravenous, topical, and pulmonary delivery of nanocarriers could be promising. Nanocarrier characteristics can be tailored for the desired delivery route in order to achieve the highest efficiency. **Figure 1** represents the targeting of infected AMs and granulomas by nanocarriers administered via different routes.

The preferable administration of nanocarriers is the oral route as it is noninvasive and more convenient for the patients to bring the treatment to completion. However, the harsh conditions found in the gastric medium – low pH and highly proteolytic media – and the hepatic first-pass metabolism limit the possible formulations and result in reduced bioavailability.[33] Intravenous administration avoids the first-pass metabolism, it allows drugs to be rapidly absorbed directly into the systemic circulation and it provides a more precise control of administered dose. The fate of intravenously injected nanocarriers is governed by protein association (protein corona)[34] and clearance by the mononuclear phagocyte system (MPS). Both oral and intravenous routes are associated with systemic side effects.[35] The noninvasive topical route allows sustained release, local action, thus less systemic side effect and bypass of the hepatic first-pass metabolism. It could be favorable in case of cutaneous tuberculosis, which is a rare condition, therefore this route is less explored.[13,36]

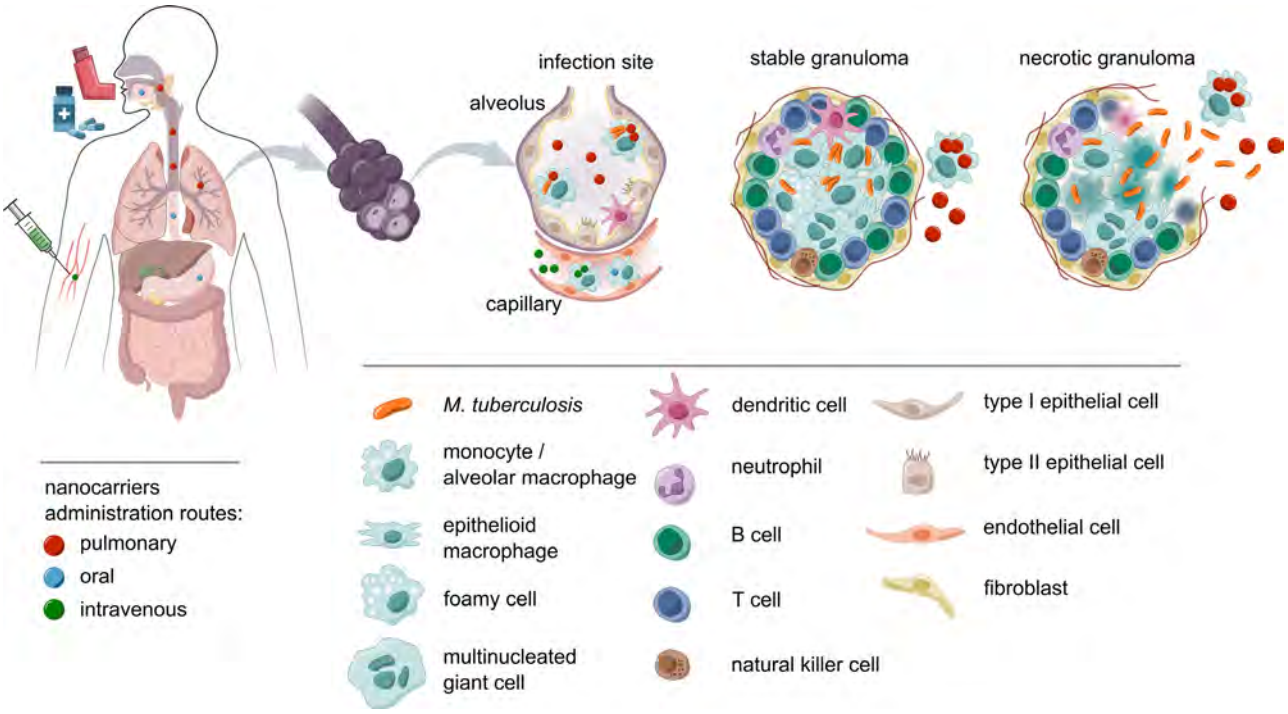

Figure 1. Targeting of alveolar macrophages and granulomas by nanocarriers administered via different routes.

Being the lung the main infected organ of pulmonary TB, its direct targeting with pulmonary administration provides the most promising approach in TB treatment. It further allows to achieve a more efficient therapy with lower administration doses and a consequent toxicity reduction as compared with the oral route.[13,37] Pulmonary administration is promising in case of anti-TB drugs that have limited potential if delivered orally, since they have low water solubility, low biodistribution, and exhibit significant side effects. Highly improved bioavailability also encourages the use of the pulmonary route since the drug-metabolizing enzyme activity is limited compared to other organs (gastrointestinal tract and liver).[13] Moreover, it can promote patient compliance since it is noninvasive and can be self-administered.[38] However, the organization and physiology of the respiratory system also contribute toward defense from pathogens. Thus, overcoming the biological and anatomical barriers in the respiratory tract can present challenges.[39]

The airways of the human respiratory system are lined with different cell types (epithelial, ciliated, goblet, secretory, and basal cells) with specialized functions. The major cell types of the airways are epithelial cells forming a monolayer with a gradual thinning from the bronchi of the upper airways to the alveoli. The mucus, a continuous layer covering the respiratory tract represents a protective coating, it is comprised of inorganic salts, proteins, glycoproteins (mucins), lipids, and water. The pulmonary surfactant layer lines the alveolar epithelium and contains phospholipids, cholesterol, and proteins. These layers affect particle dissolution, diffusion, and interaction between therapeutic agents, cell surfaces, and receptors, and they are responsible for the clearance of particles from the lungs.[40]

Alveoli, the terminating ends of all airway passages, form the functional tissue of the lung where the gas exchange occurs. The alveolar epithelium has a cellular component represented by interstitial cells, smooth muscle cells, epithelial cells (squamous type I and cuboidal type II), dendritic cells, and AMs and a connective part that includes elastic fibers, type I collagen. The systemic absorption promoted by the presence of this highly vascularized architecture can decrease the local concentration of drugs, thus this aspect could be useful to treat also extrapulmonary TB.[13,40] *Mtb* mainly resides in infected AMs or granulomas, therefore, alveoli represent the main target site of anti-TB therapeutics.

Beside the presented biological barrier, the deposition of inhaled therapeutics in the airways represents another obstacle in reaching the target cells. The deposition of aerosolized nanocarriers depend on their physicochemical properties, such as particle size, aerodynamic size, and size distribution, shape, density, and electrostatic charge.[13,40] Among them, particle size plays the most important role in achieving deep lung deposition. The inhaled particles should be small enough to avoid deposition in the upper airways by sedimentation or impaction and capable of avoiding the mucociliary clearance, but they should also avoid loss by exhalation. In general, it has been stated that large microparticles (aerodynamic diameter between 5 and 30 μm) deposit in the oropharynx, trachea, and bronchi for inertial impaction, smaller ones, from 1 to 5 μm, can reach the lower airways and can be efficiently taken up by macrophages but they tend to form aggregates due to improper aerodynamic properties and are subjected to mucociliary clearance. Finally, particles with a size lower than 1 μm are capable of reaching the alveoli through Brownian diffusion but they may be exhaled before their deposition into lungs, because of their low inertia.[41,42] To guide the nanocarriers along the upper respiratory airways, devices, such as dry powder inhalers, pressurized metered-dose inhalers, and

nebulizers, may provide a breakthrough as an alternative therapeutic approach.[13,37,40]

3.2. Passive Targeting

Passive targeting refers to the favored distribution of the drug delivery nanocarrier to the area of interest without needing the functionalization with ligands specific for receptors characteristic of that area. In the case of TB, there are two main strategies that have been used to attain passive targeting. In the first strategy, we can take advantage from the fact that macrophages, monocytes, and dendritic cells, as members of the MPS, take up nanoparticles avidly by phagocytosis or endocytosis. This issue, which is usually a problem for other applications (e.g., tumor therapy), is beneficial in the case of treating infectious diseases, as immune cells are continuously recruited to the areas of infection, particularly to granulomas. In addition, nanocarriers can be tailored for reaching enhanced uptake by MPS varying their characteristics (size, charge, rigidness, shape). For example, it is known that positively charged nanoparticles are more avidly taken up by macrophages than anionic particles, since cells are slightly negatively charged. The internalization routes will differ attending to the properties of each system, e.g., phagocytosis or endocytosis.[43] Protein corona accumulation, opsonization, also promotes phagocytosis by MPS.[34] Nanocarriers that are taken up by macrophages, are transported then to the infected area.[44,45]

The second strategy resembles the controversial enhanced permeation and retention (EPR) effect by which, as a consequence of their size, accumulation of nanocarriers is favored within the tumor microenvironment compared to other areas. This superior permeation of nanocarriers arises from the rapid growth of tumor cells which implies an angiogenesis process in which a less-defined vasculature with pores and cavities with nanometric size is produced. This phenomenon however has been set in high controversy, since it was postulated as the main driving force to produce nanoparticle accumulation in the tumor microenvironment, but only modest values of accumulation have been generally observed.[46] It has been suggested that EPR could take place in bacteria-infected tissues since inflammatory reactions also result in increased microvascular permeability of the capillaries.[47] Therefore, in principle, it would be possible to take advantage of this phenomenon to enhance activity of nanoparticles at infection sites, such as a granuloma. To do so, nanoparticles with long circulation times would be preferred so that they are more likely to accumulate in this area.

Plenty of studies demonstrate the in vitro and in vivo achievements of using passively targeted nanocarriers against TB as reviewed recently.[12,13,48–54] Most in vitro experimental models of TB infection for studying effects of nanocarriers use primary macrophages, macrophage-like immortalized cell lines, and induced pluripotent-stem-cell-derived macrophages. In vitro granuloma model based on human peripheral blood mononuclear cells (PBMCs) represents a more complex system.[55] Animal models for TB are mice, guinea pigs, rabbits, nonhuman primates, and zebra fish (adult and larvae).[56]

Some recent studies applying nanocarriers via passive targeting through nonpulmonary administration are summarized in **Table 1**.

To illustrate the state of the art in the field of TB nanotherapeutics we have selected one example that covers all the steps from the polymer design, nanoparticle characterization to in vivo evaluation. Trousil et al.[45] prepared block copolymers based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(caprolactone) (PCL) blocks, with different hydrophilic to hydrophobic ratio and molecular weights. These amphiphilic polymers are able to self-assemble in solution forming nanoparticles, encapsulating RIF in the inner hydrophobic core, with good drug loadings (from 10% to 23%). PCL is a biodegradable polyester that undergoes slow hydrolysis catalyzed by lipases in physiological conditions. Although lipase may favor the drug release, in the hydrolysis and release study, it was found that the release process was driven by diffusion from the nanoparticle core. The first step in the biological characterization attended to cell viability and internalization rate in the murine monocyte–macrophage Raw 264.7 cell line. The plain nanoparticles had no cytotoxic activity. However, encapsulated RIF was more toxic than the free drug, what can be correlated with an enhanced uptake favored by the encapsulation of the drug. The hydrophilic to hydrophobic ratio drove the uptake rate: particles with higher hydrophilic content were internalized to a greater extent which was ascribed to be less prone to develop a thicker protein corona. Among all nanoparticles, the authors selected only one, that gathered great colloidal stability, appropriate size (75 nm with 4:6 hydrophobic to hydrophilic ratio), and good in vitro performance (cell internalization and degradability). The general antibacterial properties of the nanoformulation were studied using several bacterial strains. The selected nanoparticle resulted in a 1.3-fold to sixfold reduction of the minimal inhibitory concentration (MIC) depending on the bacterial strain, which suggested that the polymer was capable of permeating the bacterial membrane. Macrophages infected with *Mtb* H37Rv strain were subsequently evaluated as a more complex model to study toxicity and internalization of the nanoformulations. After 48 h incubation with the nanoparticles, the authors observed that the encapsulated drug was just slightly more active than the free drug. However, they extended the experiment up to 5 days and found that when treated with free RIF, the infection persisted, while it was completely eradicated when the drug had been previously encapsulated. Based on that, the RIF-free nanoparticles also had some activity in the infected macrophage experiments, the authors proposed that the amphiphilic structure of the polymer interacted with the mycobacterial wall, sensitizing bacteria against the internal elimination mechanisms of macrophages. Finally, the in vivo antimicrobial activity was studied in zebra fish embryos infected by injection with *Mycobacterium marinum*. The experiments were performed with a single injected dose the day after infection. The mortality in the infected group treated with RIF was delayed for about 3 days compared with the untreated infected group. However, the survival rate was 0% after 9 days. By contrast, the administration of encapsulated drug led to an outstanding 50% survival rate 9 days after infection.[45] The pharmacokinetics, pharmacodynamics, and anti-TB efficacy of the nanoformulated RIF were further assessed in a mouse model (BALB/c mice infected intranasally with *Mtb* H37Rv) as a clinically relevant in vivo model.[64] Biodistribution study revealed that the nanoparticles were found mainly in the liver and in intestinal tissue, i.e., macrophage-rich organs. The nanoparticles

Table 1. Summary of some recent contributions of nanocarriers via passive targeting in TB treatment – in vitro and in vivo studies through nonpulmonary administration.[a)]

Nanocarrier	Loading	Key findings	Ref.
PLGA nanoparticles (NPs)	RIF, coumarin-6	Enhanced activity on intracellular bacteria (BMDM infected with *Mycobacterium bovis* BCG) compared to free drug; NPs remained membrane-bound in phagolysosomes in RAW 264.7 cells	[57]
PLA–PEG polymeric micelles	INH (conjugated), RIF	Loaded micelles were less hemolytic and had lower MIC values on *Mtb* than free drugs	[58]
PLGA NPs, alginate-coated or -entrapped	AMK, MXF	Coencapsulation of AMK and MXF performed better than each drug separately in inhibiting *Mtb* H37Ra; efficient uptake in dTHP-1 cells; slower release in phosphate buffered saline solution	[59]
Chitosan nanocapsules and lipid NPs (soybean lecithin, DOTAP, PEG 40 stearate)	BDQ	Nanoformulations had similar MIC as free drug against *Mtb* H37Rv; low or no cytotoxicity on dTHP-1, A549 and HepG2 cells; high stability and absence of drug burst release in different media and human plasma	[60]
Polypeptidic micelles, alginate coating	BDQ	Enhanced antimicrobial activity against *Mtb* H37Rv compared to free drug; enhanced stability in gastric and intestinal simulated media	[61]
PLGA NPs, methacrylic acid–ethyl acrylate-based coating	RIF	Microparticle formulation protected the NPs from degradation under simulated gastric conditions; enhanced activity in *Mtb*-infected MH-S cells; NPs were able to cross the in vitro model of intestinal barrier	[62]
PLGA NPs	RIF, thioridazine (TZ, a bacterial efflux pump inhibitor)	Encapsulation reduced toxicity of TZ in macrophages (BMDM and human monocyte-derived macrophages) and in zebra fish (Casper, Tg); enhanced therapeutic effect in *Mycobacterium marinum*-infected zebra fish; TZ–PLGA can be used as adjuvant for RIF treatment	[63]
Methoxy poly(ethylene oxide)-*block*-poly(*ϵ*-caprolactone) NPs	RIF	Cytotoxicity on RAW 264.7 cells; enhanced MICs on different bacterial strains; enhanced activity against intracellular bacteria (RAW 264.7 infected with *Mtb* H37Rv); NPs were not toxic and were more efficient in *M. marinum*-infected zebra fish; high anti-TB efficiency after intraperitoneal administration in BALB/c mice infected intranasally with *Mtb* H37Rv	[45,64]
1,8-Octanediol-dimethyl 2-oxoglutarate copolymer NPs	INH (conjugated), clofazimine (CFZ), coumarin-6	Rapid, high-level accumulation in monocytes and neutrophils, and less efficient uptake by B and T cells in human PBMC; colocalization with *Mtb* H37Rv in phagosomes of dTHP-1; in *M. marinum*-infected zebra fish model NPs were taken up by macrophages; NPs had better activity in reducing bacterial burden and granuloma number	[65]
PLGA NPs, other type of NPs	RIF, several fluorescent dyes	Rapid NP uptake by macrophages and lowered bacterial load in *M. marinum*-infected zebra fish model; NP accumulation in granulomas in zebra fish model and in C57BL/6 mice (intranasally infected with *Mtb*) following intravenous administration	[66,67]
Niosomes (Span 60, Span 85, cholesterol, dicetyl phosphate, stearylamine)	ETB	Sustained release of drug in Swiss albino mice lungs after subcutaneous injection of niosomes; enhanced efficiency in decreasing bacterial counts in lung of guinea pigs (infected by intramuscular injection of *Mtb* H37Rv) after subcutaneous injection	[68]
PLGA NPs	Ethionamide (ETH)	Enhanced bioavailability of NPs when administered orally compared to free drug; ETH detected in lung, liver, and spleen, and no toxic effects were found in Swiss albino mice	[69,70]
Chitosan–dextran sulfate nanoparticles	Aminoglycosides: streptomycin (STR), gentamicin, tobramycin	Orally administered STR NPs were as effective as subcutaneously injected aqueous STR solution in a chronic infection mouse model (BALB/c mouse infected with aerosol of *Mtb* psmt-Erdman strain)	[71]
Poly(caprolactone) (PCL) NPs	ETB, ^{99m}Tc for imaging	NPs were as effective against intracellular bacteria as free ETB in *M. bovis* BCG-infected J774A.1 cells; NPs reached the lung following intraocular injection in mice infected by *M. bovis* BCG through the thoracic cavity	[72]

[a)] Abbreviations: MIC: minimal inhibitory concentration; PLGA: poly(lactic-*co*-glycolic acid); A549: human lung alveolar epithelial cell line; BCG: Bacillus Calmette-Guérin; BMDM: murine-bone-marrow-derived macrophages; dTHP-1: differentiated human macrophage-like cells; HepG2: human liver epithelial cell line; J774: murine macrophage cell line; MH-S: murine alveolar macrophages; PBMC: human peripheral blood mononuclear cells; RAW 264.7: murine macrophage cell line; PEG: poly(ethylene glycol); DOTAP: 1,2-dioleoyl-3-trimethylammoniumpropane.

were well tolerated, compared to free RIF, RIF-loaded nanoparticles showed improved pharmacokinetic and pharmacodynamic parameters with higher serum and lung RIF level. Both free drug and drug-loaded nanoparticles reduced the bacterial burden in both lung and spleen after treatment with intraperitoneal administration. Importantly, the treatment with drug-loaded nanoparticles was preferred as significantly less granulomas were observed compared to the treatment with the free drug.[64]

Zebrafish larvae have emerged as a vertebrate animal model since they gather unique characteristics like transparency, ease of manipulation, an innate immune response, and high degree of similarities with human genome. Importantly, granulomas formed in *M. marinum*-infected zebra fish model resemble human *Mtb* granulomas to a greater extent than that of the *Mtb* in a mouse model.[73,74] These features permit to generate a robust model to screen nanocarriers before going to more complex models like mammals, that involve further ethical considerations. The Griffith group has used this in vivo model for studying anti-TB-drug-loaded liposomes and polymeric nanoparticles.[66,67,75] They showed that intravenously injected poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles were internalized by both infected and uninfected macrophages in the zebra fish larvae. Nanoparticle-containing uninfected macrophages could subsequently migrate to the granulomas providing an efficient targeting. RIF-loaded nanoparticles significantly increased survival and lowered bacterial load.[66] In order to evaluate the possible EPR effect in granulomas in infected zebra fish larvae, Fenaroli et al.[67] injected *M. marinum* in the neural tube of zebra fish instead of intravenously. This protocol for the infection, which had been introduced previously by Oehlers et al., allowed the formation of well-defined granulomas compared to less defined dynamic structures observed after intravenous injection.[76] The studied liposomes and nanoparticles, covered with stealth-like polymers (PEG or poly(sarcosine) (Psar)), crossed the endothelial barriers near infection sites within minutes after injection and accumulated close to granulomas. They found that the vasculature increased notoriously in the surrounding area of the granulomas in the neural tube, supporting this idea of the augmented vascularity.[76] In mice infected intranasally with *Mtb*, they observed the same preferential accumulation of PEG–liposomes and Psar micelles in the granuloma showing that the zebra fish model can be used to predict nanoparticle behavior in mammalian hosts.[67] Furthermore, the pharmacokinetics of liposomes and nanoparticles (circulation time, interactions with key target cells, macrophages, and endothelial cells) are similar in zebra fish and mice models according to a recent study carried out by Dal et al.[75]

Nanocarriers demonstrated an elevated and prolonged drug accumulation in lung in mice compared to free drugs when intravenous or subcutaneous administration was used.[67,68] However, direct pulmonary administration further enhances the bioavailability in lungs that favors the efficacy of the treatment and decreases the systemic adverse effects. Nanocarriers are regularly embedded in microparticles (1–5 µm) to achieve formulations with appropriate size for facilitated deep lung delivery. Once reaching the alveolar region, microparticles are expected to readily dissolve, delivering the nanocarriers which, in turn, are internalized by AMs and can release the encapsulated drug.[40,51,77,78] Some recent approaches applying passively targeted nanocarriers for pulmonary administration route are summarized in **Table 2**.

In a recent example for pulmonary delivery, Gaspar et al.[89] developed microparticles of trehalose and mannitol – two known excipients for preparing microparticles by spray drying – to encapsulate solid lipid nanoparticles (SLNs) that contained rifabutin (RFB). These SLNs were prepared using two types of glyceryl esters, glyceryl dibehenate and glyceryl tristearate and were covered with Tween 80 as a surfactant, yielding sizes of ≈100 and 200 nm, respectively, with a drug loading content of 9% and 6%, respectively. Spray drying the nanoparticles with trehalose or mannitol produced microparticles around aerodynamic diameters of 4 and 5 µm which are in the optimal size range for lung deposition. These excipients are readily soluble in water, so when the dry powder was resuspended in buffer, containing a lung surfactant, the SLNs could be recovered. The microparticulate system developed permitted to stabilize the nanoparticle formulation. In fact, when the drug release from the microparticles was evaluated, the authors concluded that despite the initial burst release, RFB followed a steady release over 24 h. The in vivo biodistribution study in mice after inhalation demonstrated that the microencapsulated RFB–SLN provided higher amounts of RFB in lung when compared with plain RFB in mannitol microspheres. In accordance with the literature, the inhaled microparticles were able to reach systemic circulation, thus liver and spleen. The inhaled drug-loaded formulation efficiently reduced the bacterial burden in lung, spleen, and liver of *Mtb*-infected mice (infection induced by intravenous injection of bacteria).[89]

In view of the publications reviewed during this section, we can conclude that passive targeting is a powerful tool to be considered when designing nanoparticles for drug delivery in infectious diseases, in particular in TB. This success relies on the accumulation of nanocarriers in granulomas by the recruitment of macrophages that phagocytosed the nanocarriers or via an alternative process that resembles EPR. The possibility of direct pulmonary delivery of nanocarriers raises further promises.

3.3. Active Targeting

Active targeting is now a key approach to direct more effectively drug-loaded nanocarriers toward the presented habitat of *Mtb* and to achieve enhanced specificity. Active targeting relies on the interaction between specific ligands on the surface of the nanocarriers and the receptors of the target cells. In many cases, this approach leads to receptor-mediated endocytosis, thereby, the intracellular drug concentration in target cells further increases. In the case of TB, to increase significantly the specificity of anti-TB-drug-loaded nanocarriers, we can take advantage of characteristic receptors of MPS cells as main host cells. Therefore, PRRs of phagocytes are straightforward candidates as target receptors. These receptors are responsible for sensing the presence of microorganisms by recognizing microbial PAMPs and consequently this interaction leads to the internalization of the pathogen. There are several subgroups of PRRs. They are classified according to their ligand specificity, function, localization, and evolutionary relationships. Based on their

Table 2. Summary of some recent in vitro and in vivo contributions of nanocarriers aiming passive targeting in TB treatment through pulmonary administration.[a)]

Nanocarrier	Loading	Key findings	Ref.
Superparamagnetic iron oxide NPs in gelatin and BSA-based microparticles	6-Amino-2,9-diarylpurine derivative	Multifunctional microparticulate dry powder with magnetic responsiveness; drug release is tuned by alternate magnetic fields; no cytotoxicity on L929, BMDM, and dTHP-1 cells	[79]
PLGA microspheres	Degradex near-infrared fluorescent dye	Comparison of inhalation techniques: oropharyngeal aspiration and intratracheal instillation using Microsprayer Aerosolizer or BioLite Intubation System in Swiss Webster mice; oropharyngeal aspiration showed microsphere deposition in the oral cavity; intratracheal instillation techniques resulted in the most efficient deposition in the lungs	[80]
Chitosan NPs, lactose preblend	BDQ	Dry powder formulation; no cytotoxicity on J774 cells; no toxicity in Wistar rats, highest accumulation in lungs, least concentration in blood as compared to inhaled free BDQ or orally administered BDQ solution	[81]
Poly(β-cyclodextrin) NPs	ETH and its booster	Coencapsulated formulation had improved activity against intracellular bacteria on RAW 267.4 cells infected with *Mtb* H37Rv; effective reduction of bacterial burden with subtherapeutic doses in BALB/c mice (intranasally infected with *Mtb* H37Rv) after intratracheal aerosol administration (Microsprayer)	[82]
Chitosan NPs, mixed with lactose	Prothionamide (PTH)	Better pharmacokinetic profile than free PTH; maintained drug concentration in lungs above MIC after administration of formulation with dry powder inhaler delivery device via intratracheal route in Wistar rats	[83]
Chitosan NPs, mixed with mannitol and leucine	RIF, INH	Coencapsulated dry powder formulation showed increased bioavailability for both drugs in lungs, and other macrophage-rich organs (spleen, kidney, liver); effective reduction of bacterial level after pulmonary administration with a compressor nebulizer system in BALB/c mice (infected with *Mtb* H37Rv through aerosol route)	[84]
BSA-based nano- and microparticles	STR sulfate	Lower systemic (nontarget organ) drug exposure compared to intramuscular injection and higher local drug accumulation compared to free drug in Wistar rats after using dry powder insufflator	[85]
PLGA NPs, alginate-based nanoparticles with chitosan	INH, RIF, PZA	Coencapsulated formulations had enhanced bioavailability compared to oral and intravenous administration of free drugs; it had sustained therapeutic drug levels in plasma and lungs; no hepatotoxicity was observed, after pulmonary administration (face mask connected compressor nebulizer system); no detectable bacterial load was observed in the lungs of infected guinea pigs (Dunkin Hartley, infected intramuscularly with *Mtb* H37Rv); it allowed reduced dosing frequency compared to oral administration	[86–88]
Solid lipid nanoparticles (SLNs) in trehalose or mannitol microparticles	RFB	SLNs could be recovered in lung surfactant containing buffer; enhanced lung accumulation in BALB/c mice following dry powder inhalation; NPs could reach also liver and spleen; efficient reduction of bacterial burden in lung, spleen, and liver of infected mice (infected intravenously with *Mtb* H37Rv)	[89]

[a)] Abbreviations: L929: mouse fibroblast cell line; BSA: bovine serum albumin.

localization in the cell, PRRs are divided into two main groups: membrane-bound PRRs, such as Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), and cytoplasmic PRRs, such as nucleotide-binding oligomerization domain (NOD)-like receptors and retinoic acid-inducible gene I (RIG-I)-like receptors. For receptor targeting, membrane-bound PRRs have a greater importance. TLRs can recognize mainly lipopolysaccharides, lipoproteins, heat shock proteins, and flagellar proteins, while CLRs have specificity for carbohydrate derivatives expressed on the surface of microbes (e.g., mannose, fucose, β-glucans, glycolipids).[90–93] The scavenger receptor superfamily, a subset of membrane-bound PRRs, is also a promising candidate for targeted drug delivery since they are highly expressed on macrophages. Scavenger receptors can recognize a broad variety of ligands, such as polyionic molecules including oxidized lipoproteins, phospholipids, proteoglycans, polysaccharides, and components of

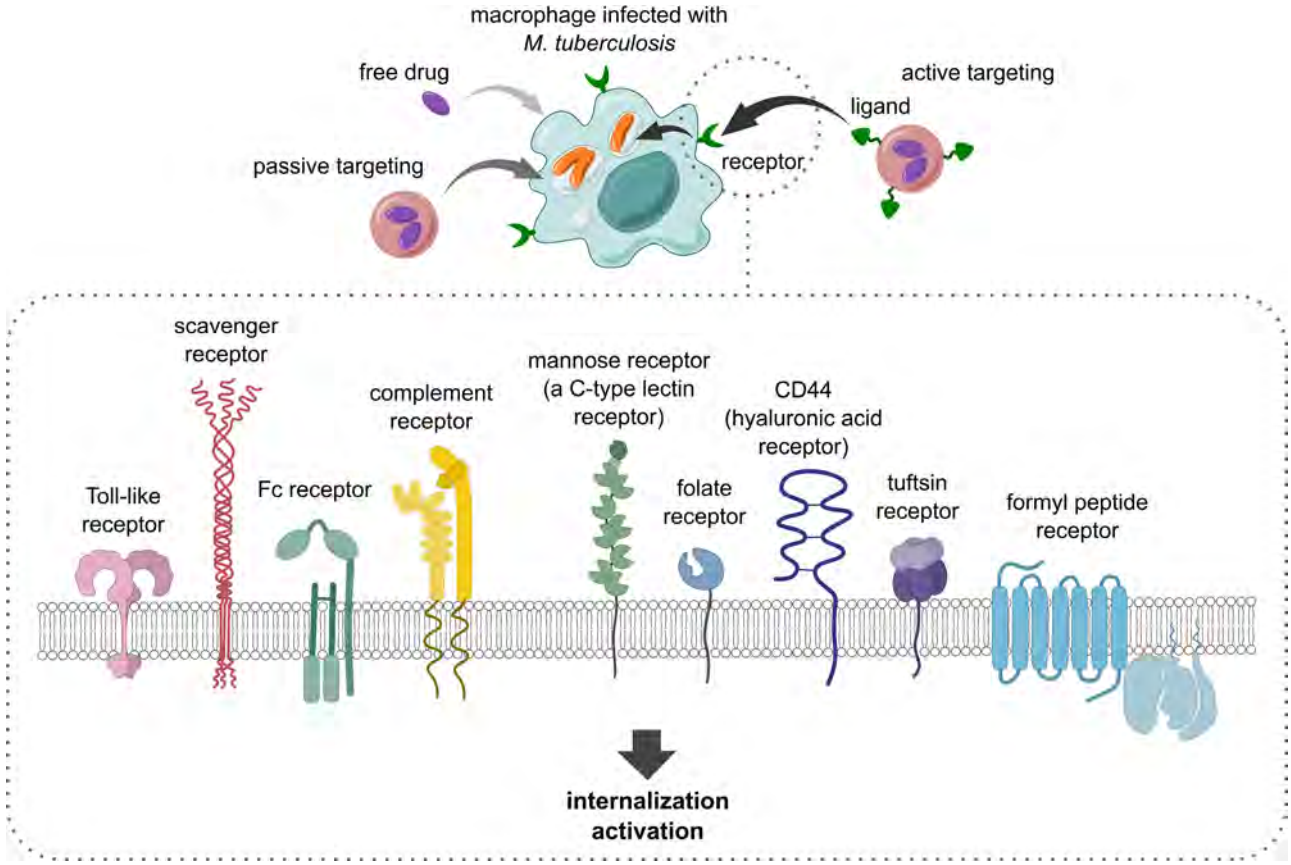

Figure 2. Drug delivery strategies to targeting infected macrophages with a schematic summary of macrophage receptors that can be used for active targeting.

bacteria.[94] Scavenger receptors play an important role in the clearance function of AMs.[95] Besides the PRRs, Fc receptors (FcR) and complement receptors (CRs) are two main phagocyte receptors and they are responsible for opsonin-dependent phagocytosis and different immune responses leading to the clearance of the opsonized pathogen. FcRs recognize the Fc fragment of antibodies, while CRs recognize complement proteins that are engaged in pathogen opsonization.[93,96,97] **Figure 2** represents the concept of active targeting of nanocarriers compared to passive targeting and the use of free drugs, and it provides a schematic summary of macrophage receptors that can be used for active targeting.

Plenty of ligands have been identified and investigated for promoting active targeting of nanocarriers. Such targeting ligands can be, among others, antibodies, proteins, peptides, carbohydrates, nucleic acid aptamers, and small molecules.[98,99] Despite that the majority of the studies of ligand-targeted nanocarrier systems are focused on cancer therapy, this knowledge can be adapted in host-cell-targeted drug delivery against intracellular pathogens, such as *Mtb*. Specifically, macrophages, besides being common host cells for intracellular pathogens, are widely studied as therapeutic target cells since they are extensively involved not only in cancer but also in inflammatory diseases.[100,101] In this section, achievements in the field of host-cell-directed targeting in the treatment of TB are highlighted.

3.3.1. Mannose Receptor Targeting

The mannose receptor (CD206) is a C-type lectin receptor that recognizes ligands with a terminal mannose, *N*-acetylglucosamine, or fucose moiety.[102] This receptor is expressed abundantly by most tissue macrophages and also by dendritic cells. It mediates endocytosis, phagocytosis, and also inflammatory signaling pathways. Moreover, it plays an important role in the uptake of *Mtb* by phagocytosis, in the inhibition of phagosome–lysosome fusion, and therefore, in the intracellular survival of the bacteria.[102–104] In addition, the mannose receptor also plays a role in the formation of granulomas.[102] Based on these findings, targeting the mannose receptor is promising for treatment of TB and intracellular colocalization of the bacteria and the nanocarrier is more possible as they can share the same entry pathway into the macrophages. A great number of works have focused on targeting macrophages through their mannose receptor and demonstrated improved uptake of the mannosylated nanocarriers relative to nontargeted formulations. Different functionalization strategies were developed for mannosylation including the synthesis of mannose–nanocarrier building block conjugates followed by nanocarrier formation, covalent functionalization of the formed nanocarriers, and physical adsorption of mannose to the surface of the nanocarriers.[105,106] Recent findings on active targeting of anti-TB-drug-loaded nanocarriers via macrophage mannose receptor are summarized in **Table 3**.

Table 3. Examples of active targeting via mannose receptor in nanotechnology-based anti-TB drug delivery systems.[a)]

Nanocarrier	Loading	Ligand, method	Key findings	Ref.
SLN: palmitic acid, cholesteryl myristate	RIF	Mannosylated fatty acid derivatives	Inhalable powder; enhanced cellular uptake on J774 cells; lipid corona formation did not modify uptake on MH-S cells; highest retention in lungs, slight inflammatory response, low systemic biodistribution in Swiss albino mice (intratracheal instillation)	[107–109]
SLN: Witepsol E85 (hydrogenated coco-glycerides), stearylamine, PVA as surfactant, inner aqueous phase	INH, coumarin-6	Stearylamine–mannose derivative by formation of Schiff base	Reduced cytotoxicity of INH on NCI-H441 and on dTHP-1 cells; enhanced uptake on dTHP-1	[110]
SLN: glycerol tripalmitate, stearylamine, Tween 80 as surfactant	RIF, coumarin-6	Stearylamine–mannose derivative by formation of Schiff base	Slightly reduced cytotoxicity of RIF on dTHP-1 cells; slightly enhanced uptake on dTHP-1; energy-dependent, clathrin-mediated endocytosis	[111]
SLN: tristearin, soya-lecithin, stearylamine, Tween 80 as surfactant	RFB, FITC	Stearylamine–mannose derivative by formation of Schiff base	Enhanced uptake on J774, prolonged circulation, high lung accumulation in Sprague-Dawley rat (i.v. injection)	[112]
Gelatin NPs (glutaraldehyde cross-linked)	Rhodamine B isothiocyanate, Texas Red	Formation of amide bond between 1-aminophenyl-α-D-mannopyranoside and gelatin, with EDC, NHS	No cytotoxicity on J774E; enhanced uptake on J774E; internalization via the mannose receptor; in albino mice (i.v. injection) organs with phagocytic cells internalized NPs in a higher extent (liver, spleen), low heart accumulation	[113]
NLC: Precirol ATO 5 (glyceryl palmitostearate), miglyol-812 (caprylic/capric triglyceride), stearylamine, Tween 60 as surfactant	RFB, RIF, FITC	Stearylamine–mannose derivative by formation of Schiff base	Slight cytotoxicity on lung epithelial cells (Calu-3, A529) and macrophages (RAW 264.7, BMDM); enhanced internalization by BMDM; more efficient restriction of intracellular growth of *M. avium* in infected BMDM	[114,115]
NLC: ovolecithin, medium chain triglyceride, octadecylamine, dimethyldioctadecyl-ammonium bromide	RIF, FITC–DHPE	Mannosylated cholesterol derivative	Enhanced uptake on NR8383 cells; low toxicity and no inflammatory response in L929 cells; enhanced cellular uptake by AMs in Wistar rats (i.v. injection); no inflammatory response; superior lung targeting of NLC compared to high RIF accumulation in liver in case of free RIF in ICR mice (i.v. injection)	[116]
PEGylated graphene oxide nanocarriers	RIF, coumarin-6, 3,3′-diethylthiadicarbocyanine iodide, doxorubicin	Formation of Schiff base between mannose and amino-PEG	Good activity on extracellular bacteria; very low cytotoxicity; enhanced uptake on dTHP-1 cells; enhanced ex vivo cellular uptake and selectivity on primary macrophages and intraepithelial lymphocytes from intestinal mucosae of *Mtb*-infected Rhesus monkey; mannose-receptor-mediated endocytosis and macropinocytosis/phagocytosis, lysosomal localization; improved activity on intracellular bacteria in infected dTHP-1 and infected monocyte-derived macrophages from healthy Rhesus monkey	[117]
Chitosan-stabilized selenium NPs (Na_2SeO_3)	INH, coumarin-6, 3,3′-diethylthiadicarbocyanine iodide	Mannose reacted with the amino group of chitosan with the aid of EDC	Low cytotoxicity on dTHP-1 and HLMVEC; synergistic activity on extracellular bacteria; enhanced uptake and selectivity toward macrophages; enhanced ex vivo cellular uptake and selectivity on primary macrophages and intraepithelial lymphocytes from intestinal mucosae of Rhesus monkey; mannose-receptor-mediated endocytosis, clathrin-mediated endocytosis, nanoparticles colocalized in lysosomes with *Mtb*; improved activity on intracellular bacteria in infected dTHP-1 and infected monocyte-derived macrophages from healthy Rhesus monkey; NPs promote the fusion of *Mtb* into lysosomes, enhance autophagy and apoptosis, regulate macrophage polarization and cytokine production	[118]

(*Continued*)

Table 3. Continued

Nanocarrier	Loading	Ligand, method	Key findings	Ref.
Gelatin NPs (glutaraldehyde cross-linked)	INH, FITC	Formation of Schiff base between mannose and gelatin	Enhanced uptake on J774; effective delivery of NPs to alveolar tissues; reduced hepatotoxicity; enhanced anti-TB activity on BALB/c mice (intranasally infected with *Mtb* H37Rv) after NP i.v. injection	[119]
Polymeric micelles modified with chitosan and hydrolyzed galactomannan, PCL-*b*-PEG-*b*-PCL	RIF	Mannose from hydrolyzed galactomannan	Enhanced uptake on RAW 264.7 cells	[120]
Liposomes, microparticles: L-α-phosphatidylcholine, cholesterol, phosphatidyl-ethanolamine (PE), cospray drying with dextran as carrier	MXF, FITC	4-Aminophenyl-α-D-mannopyranoside, PE amino group, glutaraldehyde cross-linking	Liposomes had same MIC as free MXF on extracellular *Mtb*; low cytotoxicity on A549 cells; enhanced uptake on J774A.1 cells; mainly alveolar localization of ungrafted formulation after intrapulmonary administration with dry powder inhaler in albino rats (mannosylated was not tested)	[121]
Liposomes: DSPC, cholesterol	FITC–DHPE	Mannosylated cholesterol derivative	Enhanced uptake on primary rat AMs; in vivo selectivity: higher association with AMs, rather than alveolar epithelial type II cells in Wistar rats after intratracheal administration	[122]
Liposomes: hydrogenated egg yolk phosphatidylcholine, cholesterol, dicetylphosphate, [^{3}H]cholesteryl-hexadecylether	5(6)-Carboxyfluorescein	4-Aminophenyl-α-D-mannopyranoside	Enhanced uptake on NR8383 cells; in vivo enhanced delivery to AMs; no pulmonary toxicity in Sprague-Dawley rats after intrapulmonary administration using liquid Microsprayer aerosolizer	[123]
Nanocarrier assembly: hydrophobic core–hydrophilic corona structure, PS-*b*-PEG–mannose block copolymer	EtTP5 fluorophore	Mannosylation of PEG by copper(I)-catalyzed azide–alkyne cycloaddition	Cellular uptake on J774E, J774A.1 cells; determination of optimal mannose density on the surface of nanocarriers	[124]
Chitosan nanocapsules, Tween 20, Span 85, oleic acid	Nile Red, DiD flurophores	Trimannose ligand, BS^3 cross-linker	Similar uptake of grafted and nongrafted nanocapsules on differentiated macrophages; actin cytoskeleton-dependent process; grafted nanocapsules remodeled the response of *Mtb*-infected macrophages	[126]
Liposomes: DMPC, cholesterol, stearylamine	Amphotericin B, Nile Red	Palmitoyl mannose or 4-SO_4GalNAc	Enhanced uptake of 4-SO_4GalNAc–liposomes compared to mannosylated liposomes on J774A.1 and RAW 267.4 cells, no cytotoxicity on these cells; 4-SO_4GalNAc strongly interacts with the cysteine rich domain of the mannose receptor; functionalized liposomes had higher accumulation in liver and spleen of Wistar rats after i.v. administration	[127]

[a] Abbreviations: i.v.: intravenous; NLC: nanostructured lipid carrier; A529: human bronchial epithelial cell line; Calu-3: human airway epithelial cell line; HLMVEC: human lung microvascular endothelial cells; NCI-H441: human lung epithelial cell line; NR8383: rat alveolar macrophage cell line; SLN: solid lipid nanoparticle; PVA: poly(vinyl alcohol); FITC: fluorescein-isothiocyanate; EDC: 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide; NHS: *N*-hydroxysuccinimide; DHPE: 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine; DSPC: 1,2-distearoyl-*sn*-glycero-3-phosphocholine; PS: poly(styrene); DMPC: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine.

Inhalable, RIF-loaded mannosylated SLNs were developed to target AMs by dry powder inhaler device. Mannosylated fatty acid derivatives with a dual role of functionalizing agents and surfactants were incorporated into SLNs. The improved targeting ability was verified in vitro, mannosylated SLNs exhibited higher cellular uptake rate as compared to RIF and nonfunctionalized SLNs. The functionalized particles also had an adequate respirability that is promising for the future use in inhalation therapy.[107] Furthermore, using a pulmonary surfactant model, it was shown that the lipid corona formed around SLNs improved drug retention in simulated lung fluid. The presence of the lipid corona did not mask completely the functional groups on nanoparticle surface, since the in vitro uptake rate did not decrease significantly.[108] Importantly, in in vivo biodistribution studies in mice after intratracheal instillation, mannosylated SLNs showed the highest retention in lungs associated with a poor spreading in extrapulmonary regions in comparison with both nonfunctionalized SLNs and bare RIF.[109]

Costa et al. prepared stearylamine containing SLNs using hydrogenated coco-glycerides as lipid components. The SLNs were functionalized with mannose forming a Schiff base (imine) between the primary amine group of stearylamine and the aldehyde group of the open-chain form of mannose. These SLNs loaded with INH were able to reduce the in vitro cytotoxicity of INH on lung epithelial cells and macrophages. Mannosylation enhanced the in vitro uptake of fluorescently labeled SLNs by

macrophages.[110] Similar results were obtained with RIF-loaded mannosylated SLNs composed of glycerol tripalmitate as lipid component.[111]

Mannosylated SLNs composed of tristearin, soya lecithin, stearylamine and loaded with RFB were prepared, and this formulation was found to be in the highest level in lungs as compared to the free RFB and the uncoated SLN in in vivo experiments with rats after intravenous injection. The free RFB had the highest concentration in the kidney and it had a rapid clearance by this organ. Moreover, the study suggests that mannosylation possibly helped to bypass renal elimination and that mannosylated SLNs are rather eliminated by the liver.[112] Other in vivo results also confirmed that mannose coating has advantageous influence on the biodistribution of nanocarriers. Gelatin nanoparticles were internalized to a greater extent in organs with phagocytic cells, such as liver and spleen, while low accumulation in the heart was observed. Mannosylation further enhanced the accumulation in liver and spleen.[113]

Nanostructured lipid carriers (NLCs) are composed of a mixture of solid and liquid lipids, and these nanoparticles showed enhanced drug-loading capacity compared to SLNs. Unloaded NLCs showed a slight in vitro cytotoxicity on human airway epithelial and bronchial epithelial cell lines, and on murine macrophages, however, mannosylated RIF-loaded NLCs had improved cellular uptake and showed better intracellular growth inhibition of *Mycobacterium avium* in infected macrophages than free RIF or nonfunctionalized NLCs at noncytotoxic concentrations.[114,115] Other mannosylated, RIF-loaded cationic NLCs displayed superior lung-targeting ability in vivo after intravenous administration without inducing inflammatory response in mice and rats. Greater AM uptake of mannosylated NLCs was observed in vitro and in vivo compared to unmodified NLCs. Furthermore, the in vivo inhibition of mannose receptor in rats by intratracheal instillation with mannan dramatically decreased the uptake of mannosylated NLC, demonstrating the involvement of the receptor in the uptake. The authors also proposed that these cationic NLCs interact with serum proteins, thus generating large and loose complexes which can lead to their specific accumulation in the lung.[116]

In a recent study, mannose-surface-decorated and PEGylated graphene oxide nanocarriers have been shown to enhance the uptake of RIF and also remarkably improved elimination of intracellular *Mtb* in in vitro and ex vivo models. For these nanocarriers, the predominant cellular uptake pathways were mannose-receptor-mediated endocytosis and micropinocytosis or phagocytosis. The internalized nanocarriers were mainly accumulated in the lysosomes, where the acidic environment could accelerate the release of RIF to kill the intracellular *Mtb* more efficiently. Additionally, nanocarrier treatment maintained a much higher intracellular RIF concentration in macrophages in vitro as compared to the free drug. In ex vivo experiment using intraepithelial lymphocyte isolated from the intestine of *Mtb* H37Rv-infected Rhesus monkey, it was determined that the nanocarrier was internalized in remarkably higher extent by CD14+ primary macrophages, the uptake by macrophages was nearly double than the uptake by epithelium and CD14− lymphocytes. This finding demonstrated the selective targeting potential of mannosylated nanocarriers against macrophages.[117] This group also synthesized mannosylated, chitosan-stabilized INH-loaded selenium nanoparticles with core–shell structure. With these nanoparticles, similar results (including enhanced cellular uptake, intracellular killing of *Mtb*, and selectivity in in vitro and ex vivo models due to mannosylation) were achieved. Unloaded nanoparticles inherently inhibited bacteria and the INH-loaded nanoparticle showed synergistic bacterial killing. Moreover, the nanoparticles may also contribute to the innate immunity of the infected host cells since they promote the fusion of *Mtb* containing phagosomes with lysosomes, thus synergizing the lysosomal and INH driven destruction of intracellular bacteria. Furthermore, autophagy and apoptosis are also involved in the nanoparticle-mediated intracellular antibacterial activity. Altogether, these nanoparticles achieved a highly effective intracellular *Mtb* clearance in vitro.[118]

In case of INH-loaded mannosylated gelatin nanoparticles, effective delivery of mannosylated nanocarriers to alveolar tissues was observed accompanied by reduced hepatotoxicity. Importantly, these nanoparticles possessed an enhanced in vivo anti-TB activity in *Mtb*-infected mice.[119]

Hydrolyzed galactomannan providing mannose residues was used as targeting moiety. RIF-loaded polymeric micelles surface-decorated with chitosan and combined with hydrolyzed galactomannan boosted intracellular levels of RIF in murine macrophages.[120]

For improved physical stability and lung deposition, liposomes loaded with MXF were embedded into microparticles using dextran as a carrier by spray drying. Mannosylation of the liposomes remarkably enhanced the in vitro uptake by AMs. The in vitro anti-TB activity of MXF was preserved in the formulations. Deep lung deposition was confirmed in rats by intrapulmonary administration using a dry powder inhaler. However, it was not tested with the mannosylated formulation.[121]

Wijagkanalan et al. demonstrated in vivo the efficient targeting of mannosylated liposomes via mannose-receptor-mediated endocytosis to AMs by intratracheal administration in rats. The liposomes had selective targeting ability, although both bare and mannosylated liposomes were preferentially associated with AMs, the mannosylated liposomes showed extensive uptake by AMs, rather than by alveolar epithelial type II cells.[122] Similarly, Chono et al. also found that mannosylated liposomes were more efficiently delivered to AMs than nonmodified liposomes after pulmonary aerosolization and liposomes did not harm the lung tissues of rats and were biocompatible in lung. They showed that the in vitro uptake of mannosylated liposomes is saturated with the increase of the surface mannosylation rate.[123] Other studies also showed that there is an optimal mannose density on the surface of nanocarriers for the maximum targeting and uptake by mannose receptor.[124] Optimal mannose units per nanocarrier building blocks and distance between adjacent mannose units are also important aspects for the efficient mannose-receptor-mediated endocytosis.[125]

In addition to targeting, mannosylation can have an impact on macrophage response as it was found in case of trimannose-grafted chitosan nanocarriers. These nanocarriers remodeled the macrophage response to bacterial infection, in particular affecting the regulation of many metabolic pathways including oxidative phosphorylation and sugar metabolism.[126]

A new mannose receptor targeting strategy was proposed with an alternate approach. A sulfated sugar ligand (4-sulfated

Table 4. Examples of active targeting via folate receptor in nanotechnology-based anti-TB drug delivery systems.

Nanocarrier	Loading	Ligand, method	Key findings	Ref.
Nanocapsules: oleic acid, Tween 80 (emulsifier), ethanol (drug cosolubilizer), chitosan oligosaccharide lactate	RIF, FITC	Folic acid conjugated to chitosan, with EDC	No cytotoxicity, enhanced uptake on NR8383 cells; aerosolization (Omron nebulizer), higher lung drug content; reduced plasma drug concentration in Sprague-Dawley rats after intrapulmonary administration using Microsprayer aerosolizer	[137]
Liquid-crystalline folate nanoparticles (self-assembled structure), NaOH, $CaCl_2$, $ZnCl_2$, hydroxypropyl methyl-cellulose	RIF	Folic acid-based nanocarrier, no conjugation	Low cytotoxicity on NR8383 cells; sustained release in 0.8% normal saline media	[139]

N-acetyl galactosamine, 4-SO_4GalNAc) of the mannose receptor was used with the aim to target infected macrophages in leishmaniasis. Coating of amphotericin B-loaded liposomes with this ligand led to an enhanced cellular uptake on macrophage cell lines compared to palmitoyl mannose modified liposomes.[127]

3.3.2. Folate Receptor Targeting

Folic acid is an essential vitamin. Its derivatives are required for single carbon transfer reactions, among others, for the biosynthesis of nucleotide precursors in mammalian cells. Folate receptors mediate the uptake of folic acid derivatives into cells by endocytosis. Among the different isoforms of folate receptors, folate receptor β is overexpressed on the surface of activated macrophages that are involved in inflammatory and autoimmune diseases, and also on tumor-associated macrophages (TAMs).[128–130] In addition, folate receptor α is upregulated in many types of cancer cells.[130–132] Therefore, folic acid and folate derivatives have been widely used as promising ligands to selectively deliver imaging and therapeutic agents to cancer cells, TAMs, and activated macrophages in inflammatory diseases.[130–133] However, less studies have focused on the potential of macrophage targeting with folate functionalized nanocarriers in the field of intracellular infections. Such examples are the folic acid-decorated antiretroviral drug nanoformulations that displayed enhanced uptake by monocyte-derived macrophages and enhanced antiretroviral activities compared with nontargeted particles. With the targeted formulations, favorable pharmacokinetic and biodistribution profiles were achieved following intramuscular administration.[134,135] As other example, in a murine model of pulmonary *Pseudomonas aeruginosa* infection, folic acid-decorated, MXF-loaded α-cyclodextrin-based nanoparticles showed better antibacterial efficacy with a prolonged survival time than the free drug and nontargeted nanoparticles.[136]

Regarding TB, targeting the folate receptor has not been a well-exploited approach so far. The findings of two studies are summarized in **Table 4**. Recently, Shah et al. demonstrated promising results in the targeted delivery of anti-TB-drug-loaded nanocarriers. RIF-loaded oleic acid-based nanoemulsion with chitosan–folate coating was prepared for pulmonary delivery. This nanoemulsion was nebulized and the obtained aerosol provided optimal characteristics for deep lung deposition. The nanoemulsions had no cytotoxicity in vitro on AMs and the chitosan–folate-coated nanoemulsions had better cellular uptake than the chitosan-coated nanoemulsions without folate modification. It is presumed that cell internalization was facilitated by both folate and mannose receptors (since chitosan has *N*-acetylglucosamine residues that can be recognized by mannose receptors). Moreover, the folate-grafted formulation led to a higher lung drug content in vivo and reduced plasma drug concentration as compared to nonfunctionalized formulations.[137] Similar approach was applied successfully using folate-grafted chitosan coating for SLNs to reach lung tissues by inhalation, although the objective of the work was focused on lung cancer therapy and not TB.[138]

In another study with the aim of developing better therapeutic approach against TB, folate had dual role: targeting ligand and building block of a self-assembled structure, namely liquid-crystalline folate nanoparticles. RIF intercalated between or within the ordered folate stacks of the nanoparticles, and an efficient RIF loading was achieved with sustained release. Cellular uptake of this construct, however, was not adequately characterized.[139]

3.3.3. Hyaluronic Acid Receptor Targeting

Hyaluronic acid (hyaluronan, HA) is a glycosaminoglycan consisting of repeating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine.[140] HA is a major component of the pericellular and extracellular matrix (ECM), it is present in the tissues all over the body and indicates healthy or inflamed conditions upon its interaction with immune cells. The high-molecular-weight HA (>1000 kDa) predominates in healthy tissues and it has immunosuppressive and anti-inflammatory properties, while low-molecular-weight HA (<500 kDa) forms upon tissue damage or infection as the components of ECM become fragmented and it exhibits immunostimulatory and proinflammatory properties. During inflammatory response, activated immune cells upregulate CD44, the HA binding receptor. CD44 is highly expressed on macrophages, moreover, AMs can bind HA also during homeostatic (noninflammatory) conditions. Macrophages take up HA in a CD44-dependent manner, then it is transported to the lysosomes.[141,142] In addition, CD44 is also a macrophage-binding site for *Mtb*[143] and the bacteria can utilize HA as a carbon source for multiplication.[144] Besides CD44, several HA-binding and HA-interacting receptors were also found, including TLR2 and TLR4 that are highly expressed on macrophages. HA fragments induce inflammatory gene expression (chemokine and cytokine expression) in macrophages through TLR2 and TLR4.[140,141] HA is biocompatible, biodegradable and has several modification sites that makes it an attractive

Table 5. Examples of active targeting via hyaluronic acid receptor in nanotechnology-based anti-TB drug delivery systems.[a)]

Nanocarrier	Loading	Ligand, method	Key findings	Ref.
HA-α-tocopherol succinate-based self-assembling micelles	RIF, Alexa 488	HA-based nanocarrier, ethylenediamine, EDC, NHS conjugation	Moderate cytotoxicity; enhanced uptake on MH-S cells; phagocytosis, CD44-mediated endocytosis; MH-S cell activation, proinflammatory cytokine and chemokine release induction	[147]
Sodium hyaluronate nanocomposite	RIF, INH, verapamil	HA-based nanocarrier, no conjugation	Spray-dried nanocomposite; low MIC values on extracellular bacteria (H73Rv, MDR, and XDR strains); low cytotoxicity on PBMC-derived macrophages; good anti-TB activity on intracellular bacteria in case of all strains	[148]
Self-assembling nanogel: HA (hydrophilic), 11-amino-1-undecanethiol (hydrophobic)	AMP LL37's analog LLKKK18 (KEFKRIVKRIKKFLRKLV) Alexa Fluor 488–HA, TAMRA–AMP	HA-based nanocarrier, EDC/NHS chemistry	Reduced cytotoxicity of AMP in nanogel form on BMDM (cytotoxic concentration is more than 23-fold higher than in case of free AMP); successful internalization by BMDM; intracellular inhibition of bacteria in BMDM infected with *M. avium* 2447 or *Mtb* H37Rv; reduced bacterial level in the lungs in C57BL/6 mice (infection by inhalation exposure) after intratracheal administration (Microsprayer aerosolizer)	[149]
Hyaluronan microspheres, blended with α-lactose monohydrate as diluent for inhalation	OFX	HA-based nanocarrier, no conjugation	Spray-dried microspheres; enhanced uptake on RAW 264.7 cells (air–liquid culture method); lower plasma concentration, higher lung accumulation in case of intratracheally administered microspheres as compared to i.v. and oral administration (OFX solution) in Sprague-Dawley rats	[150]
Hyaluronan microspheres, surfactants: mannitol, lysine, stearylamine, cetostearyl alcohol, stearyl alcohol	–	HA-based nanocarrier, no conjugation	Spray-dried microspheres; loss of cell viability of A549 cells due to the presence of the surfactant component	[151]

TAMRA: Carboxytetramethylrhodamine.

candidate to be used directly as a carrier or as targeting ligand on the surface of nanocarriers. Moreover, the immunomodulatory role of HA also could be exploited to boost strategies against infectious diseases, such as TB.[145] HA is widely studied as part of drug delivery systems not only against infections but also in cancer therapy because CD44 is also overexpressed on different cancer cells.[146] Recent findings on active targeting of anti-TB-drug-loaded nanocarriers via HA receptor are summarized in **Table 5**.

Hyaluronic acid–tocopherol succinate micelles enhanced the in vitro uptake of the incorporated RIF in AMs via phagocytosis and CD44-receptor-mediated endocytosis. These micelles could activate macrophages and trigger the secretion of proinflammatory cytokines and chemokines, thus enhancing the uptake of RIF-loaded micelles. These effects could lead to a more efficient RIF therapy against TB.[147]

Respirable particles were prepared from nanosuspension of sodium hyaluronate, RIF, INH, and verapamil, an efflux pump inhibitor. The highly respirable microparticles showed successful intracellular anti-TB activity on both susceptible and resistant *Mtb* strain-infected macrophages.[148]

Silva et al. encapsulated the LLKKK18 antimicrobial peptide (AMP) into self-assembling HA nanogels. With this formulation, the cytotoxicity of LLKKK18 was outstandingly reduced in vitro on murine-bone-marrow-derived macrophages as compared to the free AMP. The HA nanogels also enhanced the stability of the peptide by means of reduced proteolytic degradation in the macrophages. Incubation of macrophages with AMP-loaded nanogel reduced the intracellular levels of both the opportunistic *M. avium* and *Mtb* in vitro. Importantly, intratracheal administration of AMP-loaded HA nanogels with low peptide concentration significantly reduced bacterial levels in the lungs of the mice infected with *M. avium* or *Mtb*. Unloaded HA nanogels also reduced the infection levels in infected mice to a lesser extent.[149]

In a study of Hwang et al., hyaluronan microspheres containing ofloxacin (OFX) were prepared using a spray-drying method. The uptake of the microspheres was examined on an AM cell line using air–liquid culture method as a suitable in vitro model for airway drug uptake studies. OFX uptake from the HA microspheres was greater compared to plain OFX microspheres and nonformulated OFX. Importantly, the microspheres administered intratracheally resulted in a reduced systemic bioavailability and the highest lung accumulation compared to the oral and intravenous injection of nonformulated OFX solution. They presumed that the HA microspheres form a gel-like layer on the lung surface upon contact with the mucus fluid due to the mucoadhesiveness of HA that consequently provokes an enhanced uptake.[150] The aerosol performance (respirability) of HA microparticles obtained by spray drying was improved when surfactants were included in the formulations as an encouraging step to get closer to the clinical use of such HA formulations.[151]

Table 6. Examples of active targeting via tuftsin receptor in nanotechnology-based anti-TB drug delivery systems.[a)]

Nanocarrier	Loading	Ligand, method	Key findings	Ref.
PLGA NPs with Pluronic F127	INH in conjugate form, TB820 pyridopyrimidine derivative in conjugate form	INH–tuftsin conjugate, TB820–tuftsin conjugate	Drug–peptide conjugate: no cytotoxicity on PBMC, no hemolytic activity on human erythrocytes, enhanced activity on extracellular and intracellular bacteria (MonoMac6 cells infected with *Mtb* H37Rv); nanoparticle: after oral administration no toxicity in lung, spleen, liver, kidney, significantly decreased bacterial load, decreased inflammation and minimal granulomatous involvement compared to untreated control in guinea pigs (infected by intramuscular *Mtb* H37Rv injection)	[161,162]
PLGA NPs with Pluronic F127	TB515 coumaron derivative (with fluorescent property)	Oxime bond between aminooxyacetyl–tuftsin and aldehyde group of modified Pluronic F127	Enhanced cellular uptake on MonoMac6; enhanced intracellular anti-TB activity on *Mtb*-infected MonoMac6	[163]
NLC: stearic acid, oleic acid, Tween 80, hydrogenated phosphatidylcholine (Phospholipon 80H)	RIF, FITC	Oleic acid was attached to the N-terminus of tuftsin	Low cytotoxicity; improved uptake on J774A.1 cells; improved activity on extracellular bacteria	[164]
Liposomes: egg phosphatidylcholine, cholesterol	RIF	Palmitoyl tuftsin, C-terminus with fatty acyl residue through an ethylenediamine spacer arm	RIF delivered twice weekly for 2 weeks in i.v. administered liposomes was at least 2000 times more effective than the free drug in lowering the load of lung bacilli in *Mtb* H37Rv-infected Swiss albino mice (i.v. infection); lowered bacterial load in liver, spleen; increased mean survival time	[165]

[a)] Abbreviations: MonoMac6: human monocytic cell line; Pluronic F127: poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide), PEO–PPO–PEO triblock copolymer.

3.3.4. Tuftsin Receptor Targeting

Tuftsin is a natural tetrapeptide (Thr-Lys-Pro-Arg), produced by enzymatic cleavage of the Fc domain of the heavy chain of immunoglobulin G by two enzymes, a neutrophil-derived enzyme leukokininase and spleen tuftsin endocarboxypeptidase. Tuftsin and its analogs have a wide spectrum of biological activity, their wide range of immunostimulatory activity results in increased antimicrobial and antitumor properties. They can activate various components of the immune system, including macrophages, importantly they stimulate phagocytosis, pinocytosis, and chemotaxis.[152,153] Tuftsin also has effects on the nervous system.[154] Although tuftsin receptor was identified from the membrane of rabbit peritoneal granulocytes,[152,155] the mechanism of action and signaling pathway of tuftsin is still not fully understood. Monocytes and macrophages also possess specific receptors for tuftsin.[154] It was described that tuftsin also binds to neuropilin-1 that can be found in most tissues, and it plays a role, among others, in angiogenesis and axonal guidance.[156,157] Recent findings on active targeting of anti-TB-drug-loaded nanocarriers via tuftsin receptor are summarized in **Table 6**.

Several tuftsin-based carriers were developed[158] and were used for TB targeting as drug–peptide conjugates, these conjugates efficiently inhibited the intracellular bacteria in infected macrophage models.[159,160] Due to its quick biodegradation, the use of this peptide as targeting ligand is more suitable in a nanocarrier-based formulation.

In the studies of Horváti et al., INH or a drug candidate pyridopyrimidine derivative was covalently conjugated to a palmitoylated tuftsin derivative. The drug–lipopeptide conjugates were successfully encapsulated into PLGA nanoparticles stabilized with Pluronic F127 block copolymer.[161,162] For in vivo studies, infected guinea pigs were used since they are highly susceptible to infection with *Mtb* and develop the disease similarly as in humans.[56] The orally administered conjugate-loaded nanoparticles were able to decrease the bacterial level and no toxicity was observed, while in untreated control animals, progression of the disease was observed (severe lesions, parenchymal involvement, necrosis, intralesional mineralization).[161,162] This approach was further developed by grafting tuftsin derivative on the surface of a drug-candidate-loaded PLGA nanoparticle in the form of peptide–Pluronic F127 conjugate, thus enhanced cellular uptake and intracellular anti-TB activity was achieved compared to the free drug candidate and the nongrafted nanoparticles.[163] Similar in vitro results were achieved with NLCs where oleic acid-elongated tuftsin derivative was incorporated into the carrier.[164]

Drug-loaded liposomes with palmitoyl tuftsin anchored in the liposome bilayer by the long hydrophobic fatty acid part were effective in vivo not only against TB but also against infections, such as malaria, leishmaniasis, and fungal infections.[165,166] RIF delivered in these tuftsin-bearing liposomes was outstandingly more effective than the free drug or nongrafted liposomes in lowering the bacillary load in lungs of infected animals.[165]

3.3.5. Targeting of Formyl Peptide Receptors (FPRs)

FPRs (three subtypes: FPR1, FPR2, and FPR3) are key players in innate immunity, they mediate host-defense and inflammatory responses. These chemoattractant receptors are mainly expressed on innate immune cells including neutrophils,

monocytes, and macrophages, however, other cell types also express some FPRs. The first ligands defined for FPRs were *N*-formyl peptides, such as *N*-formylated methionine containing peptides (e.g., *N*-formylmethionyl-leucyl-phenylalanine (fMLF)) that are cleavage products of bacterial and mitochondrial proteins, therefore, FPRs are considered as PRRs. Subsequently, several structurally diverse ligands have been identified for these receptors.[167–169] This wide range of ligands for FPRs offers the opportunity to create a plethora of FPR targeting nanocarriers.

With fMLF-grafted liposomes, enhanced in vitro macrophage uptake and in vivo inflammatory site targeting was observed.[170] PEG-based nanocarriers modified with fMLF were developed for targeting macrophages in anti-HIV therapy.[171] In case of TB, the immune response boosting effect of FPR agonists was demonstrated. Treatment via subcutaneous route of *Mtb*-infected mice (infected intranasally or injection via lateral tail vein) with *N*-formylated peptides with or without anti-TB drugs showed immunotherapeutic potential since the bacterial load was markedly reduced in lungs and spleen as compared to the untreated mice.[172,173] Moreover, FPR1 ligand peptide–PEG conjugate was used in in vitro and in vivo granuloma models as a potential imaging biomarker for the tuberculosis granuloma.[174] These findings show the potential of macrophage targeting through FPRs in TB, however, this area is far from being exploited yet.

3.3.6. Other Receptors

Besides the presented macrophage receptors, other receptors can be also targeted. Macrophages have phenotypic heterogeneity and functional plasticity depending on their developmental origins, local environment, and disease progression.[175,176] Therefore, depending on the characteristic macrophage populations that are aimed to be targeted, a wide range of receptors can be considered, beside the ones that are presented above. Especially, in case of pulmonary macrophages, receptors – such as CD14 (lipopolysaccharide (LPS) receptor), CD200R, CD115 (colony stimulating factor 1 receptor), CD71 (transferrin receptor), Dectin-1/2 (β-glucan receptor), scavenger receptors (SR-A, CD36 (SR-B), macrophage receptor with collagenous structure (MARCO), CD163, CD204, CD68), TLRs (TLR4, TLR9), and Fc receptors (CD64, CD32, CD16) – can open up new strategies.[177] Targeting other host cells of *Mtb* also could be promising in overcoming the disease. For example, in dendritic cells, the main receptor involved in *Mtb* recognition is dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN), a C-type lectin receptor, that can be also targeted.[178,179] In addition, alongside the targeting ligands presented here, a large spectrum of other ligands were applied to target nanocarriers toward macrophages. These studies, beside tuberculosis, mainly focused on targeting macrophages in cancer and different inflammatory diseases.[101,129,180–184] In TB treatment, mycolic acids,[185] lactose,[186] fucoidan,[187] immunoglobulin G,[188] maleylated bovine serum albumin,[189] *O*-steroyl amylopectin,[189] surface ligands, or nanoparticle components proved to be promising candidates. Altogether, the so far obtained knowledge about macrophage (and other host cell) receptors and ligands provides a platform to develop novel approaches for an effective drug-delivery system.

4. Challenges and Future Perspectives

In recent decades, we have witnessed an extraordinary increase in our basic knowledge on TB. The sequencing of the first *Mtb* genome, in 1998,[190] opened the way to molecular studies from the pathogen perspective, which would result eventually in the discovery of novel drug targets and the identification of virulence factors. Diverse omics technologies have expanded vastly our current understanding of all stages of the disease, also from the host side.

In fact, tuberculosis, i.e., the "white plague" has been a real pandemic for centuries. Oldest remains of people suffering from TB have been dated 9000 years ago. Between XVII and XIX centuries, 25% of deaths in Europe were caused by TB.[191] Only during the XX century, the incidence of tuberculosis started to decline, thanks to the discovery of a vaccine and the development of a drug-based treatment, although from the 1990s, despite major medical advances, the tuberculosis pandemic raised again (an important lesson to be learnt when considering the pandemic we are living in our days), especially in the low-income countries. Poverty is hence the first challenge for tuberculosis control that will need to be addressed: up to 87% of tuberculosis global cases are located in just 25 developing countries plus the five BRICS emerging economies (Brazil, Russian Federation, India, China, and South Africa), whereas the same list of countries accounts for 34% of global COVID-19 cases. In a drastic contrast, the European region (excluding Russia) plus the USA account for 1.9% of tuberculosis and 41% of COVID-19 global cases, respectively (as of 9 July 2020).[1,192]

Nanotechnology offers a powerful tool in the fight against TB. Moreover, combining nanocarrier-based drug delivery systems with pulmonary administration route provides the most encouraging approach in TB treatment. Applying pulmonary drug delivery devices constitutes a noninvasive mode for drug administration with a bright future even for the administration of drugs for systemic action.[193] Importantly, the development of a sustainable nanomedicine-based approach that can be used with pulmonary administration is a key pillar in the control of this disease in developing countries, where the majority of the population do not access appropriate health care.[194] The nanoformulations with inhaler devices can be self-administered, thus it can give an opportunity to an easier and more accessible anti-TB drug treatment, reducing the need for complex medical equipment, medical personnel, and the overall costs of the treatment.

Despite the increasing number of publications demonstrating the advantages of respiratory administration, the lack of effective and uniform administration techniques in preclinical models generally results in poor translational success. Basically, the use of devices for respiratory administration involves the passive inhalation of the drug formulation, resulting in variations in the delivered dose. An accurate estimation of the dose delivered to the lungs is crucial since the poor delivery efficiency due to the drug loss in the reservoir, tubing of the aerosol generator, delivery accessories, and the nasopharyngeal region of the animal, can be misunderstood with a low efficacy of the treatment, and it may constitute a risk factor for development of drug resistance. For these reasons, invasive techniques, such as intratracheal instillation, are preferred to achieve higher and precise dosing of the drugs in the lungs, thus most preclinical models are markedly

different from inhalation methodologies used in humans.[80] It is crucial to consider the anatomical differences between humans and model animals of pulmonary diseases. Humans can inhale a substantial dose of aerosol from an inhaler device in a single deep breath. To correctly assess efficacy of the inhalation administration, the fabrication of high-quality devices, capable of depositing an efficacious drug dose into a the lungs of a spontaneously breathing animal is fundamental.[195] Furthermore, most of the inhaler using patients makes technical errors in inhalation, therefore, it is crucial to provide appropriate training and instructions for inhaler application.[196,197]

In recent years, there has been a continuously increasing innovation and patents filing in the development and perfection of devices for pulmonary drug delivery. Parallelly, improving and adapting the drug formulations to these devices and administration route is needed. In the following years, nano-enabled engineering of the particles and formulations will contribute to increasing the amount of drug that reaches the lungs and the stability of the formulation. In this line, patents are being filed not only on pulmonary drug delivery systems but also on their combination with old pulmonary-targeted drugs whose formulations have been adapted.[193] Undoubtedly, these innovation efforts will increase in the following years and broaden to include the simultaneous administration of anti-TB drugs via this administration route for simplification of treatment and seeking synergic effects.

Drug encapsulation pursues the improvement of antimicrobial drugs in several ways, i.e., increasing solubility, avoiding a rapid clearance, or minimizing negative side effects. However, if the nanoparticle does not contain any effect per se, it will somehow dilute the drug since it will work as an excipient. Consequently, the nanoparticle system should display high loading capacity in order to minimize the amount of material administered. We can foresee that increasing the loading of nanoparticles will result in a new generation of drug-loaded nanoparticles, aiming at lowering the dose but with better therapeutic effect. Further, techniques as microfluidics[198] and sequential nanoprecipitation[199] provide a straightforward approach to get access to high loading capacity systems. Encapsulation of hydrophilic drugs, as some second line anti-TB drugs, is challenging with many nanocarrier systems. Increasing effort to improve their encapsulation can be done by chemical conjugation via hydrolysable or responsive chemical bonds,[200] or by using systems suitable for hydrophilic drugs, such as liposomes, niosomes, or polymeric nanocapsules. Several second line anti-TB drugs have limited or no ability to penetrate cells, which impacts their efficacy and makes higher doses necessary. Their encapsulation in nanocarriers should aim at allowing the selective distribution to phagocytic cells and enhancing the uptake of the encapsulated drug by the cells. Resulting improved bioavailability will allow decreasing the dose and the severity of accompanying side effects. Moreover, improvements are particularly needed in the strategies for second line anti-TB drug encapsulation due to the rapid emergence of MDR-TB.[201]

Drug delivery applications typically require the surface modification or functionalization of the nanoparticles to improve stability, biocompatibility, incorporate targeting moieties, and thus achieve better selectivity. A wide range of strategies have been used to modify the surface of nanoparticles or functionalize nanoparticles with a variety of ligands.[202] These strategies can bear hurdles and creating more specific and complex nanoparticles with more than one functionalizing ligand is a very challenging task and it is rarely used. The chemicals that are included in the surface grafting procedures can have a significant effect on nanoparticle properties. Furthermore, precise characterization is required on the qualitative and quantitative presence of the ligands and modifying agents attached to the surface. It is important to control the surface orientation of the targeting ligands and confirm that they retain their native binding affinity for the receptor of interest. The adequate purification of these nanoparticles often encounters difficulties, resulting in the presence of residual reagents that may not have been removed during purification. Further advances in attachment strategies, conjugation efficiency, characterization, purification, and scale-up feasibility are needed to develop optimized, robust, and simple methods for such nanoparticle engineering.[202,203]

In summary, it is necessary to stress the importance of the reproducibility in the production (drug loading, encapsulation, and functionalization efficiency, physicochemical properties) of drug-loaded nanocarriers and the possibility of scaling the process. The lack of proper and specific regulatory guidelines regarding characterization, study designs, and statistical analyses represents a general obstacle in the clinical translation of nanoformulations. Optimized sharing practices are also needed to promote the translation of nanotechnology from experimental success to clinical practice.[204–209]

5. Conclusion

During the last 25 years, nanotechnology has emerged as a new strategy to improve drug delivery and efficiency of the actual treatments. However, many of the most successful nanoformulations are focused on cancer treatment and only few of them are oriented for infectious diseases. It is crucial to integrate key enabling technologies, including nanotechnology, to make a quantum leap for controlling pandemics. In this progress report, we highlighted the main challenges and potential of nanopharmaceutics for tuberculosis treatment. The improvement on the stability and solubility of active principles, and the possibility to direct the drug to a specific target make nanocarriers as a new opportunity to develop more effective treatments. Importantly, the improved bioavailability and biodistribution allows decreasing the dose and the severity of accompanying side effects. These advances also contribute to reducing the emergence of drug-resistant strains. Although it seems promising, so far nanotechnology has not provided any product in the market for this disease, and the number of patents in the field is much lower than in the case of nanomedicines for cancer. To achieve groundbreaking progress and to make the pulmonary administration of nanoparticles feasible for noninvasive clinical trials, a multidisciplinary approach is necessary: nanotechnology, medicine, and engineering must collaborate.

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Conflict of Interest

The authors declare no conflict of interest.

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