Very small lipid nanoparticles for clinical use

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Nanoparticles have attracted growing scientific interest during the last few years as an alternative material for biological and medical applications. Especially, nanotechnology could overcome some major drawbacks in the fields of diagnosis (novel and sensitive contrast agent) and/ or therapy (non viral carriers for active molecules delivery). In this context, lipid-based carriers present some advantages for industrial transfer compared to inorganic nanoparticles since they are biodegradable and not toxic for organism.

Recently, we have developed lipid nanoparticles (LNP), called Lipidots™, for the encapsulation of hydrophobic compounds such as dyes or small drugs. Manufacturing process is simple, fast, highly reproducible and clean (solvent free). The nanoparticle core is composed of a complex lipid mixture stabilised by a monolayer of phospholipids. A Pegylated coating provides them not only an excellent colloidal stability in buffer (at least 6 months at room temperature) but also stealth properties to reticulo-endothelial system after their injection *in vivo* as demonstrated by *in vivo* fluorescence imaging in mice. Ligands like saccharides, peptides or antibodies could be grafted to PEG chains in order to preferentially reach the targeted tissue. As an example, cRGD grafting around these nanoparticles facilitates their cellular internalisation *in vitro* and may promote their tissue content in tumor as observed in tumor bearing nude mice thank to the well known EPR effect, rendering them very promising for development of new strategies in cancer therapy.

The nanotoxicological assessment of these novel very small lipid nanoparticles (hydrodynamic diameter around 30 nm) is on going but we have already observed that (1) they remain stable for few hours (>15h-24h) in plasma, (2) they are very well tolerated *in vitro* (IC50~500 µg/mL in 3T3 fibroblasts, even more in Hep G2 cell line), (3) their uptake by macrophages or dendritic cells are very slow likely due to their pegylated coating and at last (4) they do not activate these dendritic cells. Moreover, they do not induce an inflammatory reaction after their systemic injection in rat as revealed by circulating cytokines dosage or complement activation studies.

Further experiments are needed in order to better understand LNP behaviour in biological fluids like the characterisation of the potential protein corona or whenever they reach biological barriers such as blood-brain, placenta, skin or intestinal barriers.