Prospects for exosomes in immunotherapy of cancer

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Abstract

Exosomes are nanometer sized membrane vesicles invaginating from multivesicular bodies and secreted from epithelial and hematopoietic cells. They were first described "in vitro" but vesicles with the hallmarks of exosomes are present in vivo in germinal centers and biological fluids. Their protein and lipid composition are unique and could account for their expanding functions such as eradication of obsolete proteins, antigen presentation or "Trojan horses" for viruses or prions. Exosome secretion could be a regulated process participating in the transfer of molecules inbetween immune cells. Despite numerous questions pertaining to their biological relevance, the potential of dendritic cell derived-exosomes as cell-free cancer vaccines is currently being assessed. This review will summarize the composition and formation of exosomes, preclinical data, Phase I trials and optimization protocols for improving their immunogenicity in tumor bearing patients.

Keywords: exosomes • immunity • immunotherapy

Introduction

Cancer treatment is an ever more challenging area. New strategies emerge such as targeted therapies by monoclonal antibodies (mAb) or anti-angiogenesis products. Some of the most promising emerging approaches involve the manipulation of the immune system [1]. Adoptive transfer (i.e. iv injection) of T cells, of antigen-loaded dendritic cells and mAb targeting FcgRIIIa brought up proofs of concept [2, 3].

As we learn more and more from cell therapies, approaches aimed at developing cell free vaccines are being developed [4]. Targeting dendritic cells (DC) in vivo using DEC205 Ab or DC-SIGN coupled to tumor antigens [5, 6] is in the current prospects. We have investigated an alternate approach of targeting i.e. the use of exosomes derived from DC which mediate immunogenicity...
by transfer of MHC/peptide complexes to naïve DC in vitro. This review presents the main biological features of exosomes, their physicochemical characteristics, discusses the potential mechanisms of exosome biogenesis, and underscores the rationale of their use for immunotherapy of cancer.

**Exosome characterization**

**Exosome genesis**

Originally, the demonstration of the exosome as a non-plasma membrane vesicle emerged with the description of the shedding of transferrin receptors during the maturation of reticulocytes into red blood cells. The release process appears to require a specific sorting into “internal vesicles” contained inside the endosomes leading to the formation of multivesicular bodies (i.e. MVBs) followed by the fusion of the limiting membrane of the MVBs with the plasma membrane. The latter fusion results in exosome release into the extracellular medium [7, 8]. Therefore, the “intraluminal vesicles” become “exosomes”. The pioneering studies in reticulocytes (which lack lysosomes) demonstrated that MVBs and their contents, by fusing with the cell surface, eliminate obsolete proteins that do not follow an intracellular degradation pathway [9, 10].

Many cell types generate exosomes in vitro. Exosome secretion was first described in hematopoietic cell types, such as reticulocytes [9, 10], B and T lymphocytes [11, 12], platelets [13], mast cells [14], and dendritic cells [4]. Other cell types also produce exosomes: tumor cells [15], intestinal epithelial cells [16], astrocytes and neurons [17].

Formation of MVBs requires protein sorting. This phenomenon involves ubiquitinylated protein-binding proteins working in sequence [18]. The endosomal sorting complex required for transport (ESCRT) 0 recognize the ubiquitinylated cargo via protein Hrs (mammalian Hepatocyte Receptor tyrosine kinase Substrate) also termed Vps (vacuolar protein sorting)-27. Hrs recruits ESCRT-I and Tsg (Tumor susceptibility gene)-101, which recruits ESCRT-III via ESCRT-II or AIP-1/Alix. The last complex drives the cargo into the budding vesicle, while the ESCRT complexes are recycled via Vps4 activity [19, 20]. A passive mechanism could also be implicated in protein sorting into the MVBs, involving the presence of membrane microdomains, tetraspanin enriched [21] or cholesterol enriched (lipid rafts) [22]. Lipid rafts are highly hydrophobic regions, which are enriched in cholesterol and glycosphingolipids. They can concentrate hydrophobic proteins, such as GPI anchored or acylated ones, and could play a role in exosome formation [23]. The budding itself seems to be dependent on the properties of a conically shaped, pro-fusogenic lipid, the lyso(bis)phosphatidic acid (LBPA), and the LBPA binding protein Alix [24]. However, Laulagnier et al. were unable to detect enrichment of LBPA in exosomes from mastocytes [23].

**Exosome composition**

The characterization of exosomes has been facilitated by their purification from culture supernatants using several procedures including differential ultracentrifugation and sucrose density flotation gradients [25]. Analyses of the purified exosome preparations have been performed using immunoelectronmicroscopy, Western Blotting, mass spectrometry (proteomics) and thin-layer chromatography (lipid composition) [26, 27].

The protein composition of exosomes analyzed by SDS-PAGE and Coomassie Blue staining differs from that of lysates from whole cells, cytosol and total membranes. Moreover, exosomes are distinct from microvesicles produced by apoptotic cells and are only secreted by living cells [26]. All the data obtained for exosomes derived from murine DC, human intestinal epithelial cells and Epstein-Barr virus (EBV) transformed B lymphocytes revealed common and cell-specific constituents. Such exosomes are highly enriched in MHC class II molecules, contained MHC class I, cytosolic chaperones (heat shock proteins), subunits of trimeric G proteins, annexins, rab proteins, integrins, adhesion molecules, enzymes, elongation factors, cytoskeleton proteins, raft-associated proteins and glycolipids. Tetraspanins are the most abundant protein family found in exosomes. Tetraspanins are membrane proteins with four transmembrane domains, involved in many different biological functions such as fusion, motility, immune stimulation, adhesion and protein sorting [28]. Several members of
### Table 1a  Identification of exosomal proteins by mass spectrometry.

| Protein name               | MW (kda) |          | Protein name               | MW (kda) |          |
|----------------------------|----------|----------|----------------------------|----------|----------|
| **Cytoskeleton**           |          |          | **Adhesion**               |          |          |
| Actin γ                    | 41       |          | Cadherin 1                 | 98       |          |
| Actinin α 4                | 104      |          | CD11a                      | 128      |          |
| Advillin                   | 92       |          | CD11b                      | 127      |          |
| β catenin                  | 85       |          | CD11c                      | 129      |          |
| Catenin E α                | 100      |          | CD13                       | 109      |          |
| Cap 1                      | 51       |          | CD166                      | 65       |          |
| Claudin 1                  | 22       |          | CD18                       | 85       |          |
| Cofilin 1                  | 18       |          | CD9                        | 25       |          |
| Coronin                    | 50       |          | Dectin 1                   | 24       |          |
| Dynamin 2                  | 97       |          | Dectin 2 β                 | 20       |          |
| Elongation factor 1α1      | 50       |          | Dectin 2 γ                 | 19       |          |
| Ezrin                      | 69       |          | Galectin 3                 | 27       |          |
| Fascin 1                   | 54       |          | Neuropilin 1               | 103      |          |
| Formin-related protein     | 122      |          | PGRL                       | 65       |          |
| IQGAP1                     | 188      |          | Pira7                      | 75       |          |
| Moesin                     | 66       |          | Plexin a1                  | 211      |          |
| Myosin heavy chain IX      | 226      |          | Plexin c1                  | 176      |          |
| Radixin                    | 68       |          | Thrombospondin 1           | 129      |          |
| Rho GDI1                   | 23       |          | Vinculin                   | 116      |          |
| Syntaxin binding protein 3 | 67       |          |                            |          | Membrane fusion |
| Syntenin                   | 32       |          | Annexin 1                  | 38       |          |
| Talin                      | 270      |          | Annexin 2                  | 54       |          |
| TCTP                       | 19       |          | Annexin 11                 | 38       |          |
| Tubulin β 5                | 50       |          | Annexin 4                  | 36       |          |
| Vimentin                   | 51       |          | Annexin 5                  | 35       |          |
Table 1a Identification of exosomal proteins by mass spectrometry.

| Protein name                                      | MW (kda) |
|--------------------------------------------------|----------|
| Annexin 6                                        | 75       |
| Annexin 7                                        | 49       |
| Arp2/3 complex                                   | 20       |
| Clathrin heavy chain                             | 192      |
| Rab10                                            | 22       |
| Rab11b                                           | 24       |
| Rab13                                            | 22       |
| Rab14                                            | 24       |
| Rab15                                            | 19       |
| Rab2, rab4, rab5a, rab5b, rab6, rab7              | 23       |
| Rab gdi α, rab gdi β                             | 50       |
| Rho a                                            | 23       |
| Rho g                                            | 21       |
| Snap 23                                          | 23       |
| Syntaxin 7                                       | 29       |
| Wd repeat containing protein 1                   | 66       |

T cell stimulation

| Protein name                    | MW (kda) |
|--------------------------------|----------|
| CD86                           | 80       |
| MHC I                          | 32       |
| MHC II                         | 28       |

Metabolic enzymes

| Protein name                    | MW (kda) |
|--------------------------------|----------|
| Transketolase                   | 66       |
| Aldehyde reductase              | 36       |
| Aspartate aminotransferase      | 46       |
| ATP citrate lyase               | 119      |
| ATPase Na+/K+                   | 115      |

Mvb formation

| Protein name                    | MW (kda) |
|--------------------------------|----------|
| Alix                           | 96       |
| Tsg101                         | 44       |
| Ubiquitin c                    | 100      |

Signaling

| Protein name                    | MW (kda) |
|--------------------------------|----------|
| 14-3-3 (β, ε, γ, ζ)             | 28       |
| Copine 1                        | 49       |
| C-src tyrosine kinase           | 50       |
| Erk2                            | 33       |
| Fyn                             | 60       |
| Gi3α                            | 40       |
| Gi2α                            | 38       |
| Grb3-3                          | 37       |
| Gs α                            | 82       |
| H-ras                           | 17       |
| Phospholipase c α               | 58       |
this family, including CD9, CD37, CD53, CD63, CD81 and CD82 are highly enriched in exosomes from virtually any cell type. In Table 1, we show the list of the major proteins found in mouse DC derived-exosomes (DEX) [26, 27, 29, 50].

The specific role of many proteins found in exosomes or involved in exosome formation have not yet been defined. For some, however, the roles are becoming clearer. For example, exosomes contain enriched fractions of phospholipase D2, and PLD2 activity has been related to exosome release [30]. Likewise, the GPI-anchored proteins CD55 and CD59 which are associated with exosomes, appear to

| Protein name               | MW (kda) | Family            |
|----------------------------|----------|-------------------|
| ULBP-1                     |          | NKG2D ligand      |
| MICA/B                     |          | NKG2D ligand      |
| ICAM-1 (t cell stimulation)|          | Ig superfamily    |
| CD81                       |          | Tetraspan         |
| CD82                       |          | Tetraspan         |
| CD62                       |          | Tetraspan         |

Table 1a Identification of exosomal proteins by mass spectrometry.

| Protein name | MW (kda) | Family            |
|--------------|----------|-------------------|
| Rap2b        | 20       |                   |
| Shps-1       | 56       |                   |
| Stat1        | 83       |                   |
| Cyclophilin a| 17       |                   |
| Cyclophilin b| 22       |                   |
| H2-m         | 28       |                   |
| Hsc 70       | 70       |                   |
| Hsp84        | 84       |                   |
| Ia associated invariant chain| 24   |                   |
| Tcp-1 (β, δ, ε and ζ) | 58   |                   |
| Tcp-1 τ      | 59       |                   |
| CD98         | 58       |                   |
| Complement factor 3 | 186 |                   |
| EEF2         | 95       |                   |
| Ferritine light chain 1 | 20 |                   |
| Ferritine light chain 2 | 20 |                   |
| Histone 1    | 14       |                   |
| Histone 2    | 13       |                   |
| Histone 3    | 13       |                   |
| Mfge-8       | 51       |                   |
| Mvp          | 96       |                   |
| Nicastrin    | 78       |                   |
| Proteasome 26s subunit3 | 49 |                   |
| Ribosomal protein 118 | 21 |                   |

Table 1b Antibody detection of other exosomal proteins.
protect them from complement attack. Another example includes the identification in exosomes of proteins that are part of the machinery involved in the biogenesis of MVBs. Thus, DEX contain Tsg101 and Alix which are components of the conserved machinery that selects ubiquinated cargo proteins for sorting to intraluminal vesicles [24]. The role of the most abundant proteins found on DEX, such as MHC class I, II, CD86 molecules, ICAM-1/CD54, a milk fat globule protein MFG-E8/Lactadherin, for eliciting antigen-specific T cell immune responses is discussed below.

The lipid composition of exosomes from DC and mast cells was reported in 2004 [23] and showed that their lipid composition differed from any other DC and mast cell membranes, in that they are enriched in some lipids (sphingomyelin, lysophosphatidylcholine LPC, saturated fatty acid), and relatively poor in some others (phosphatidylcholine, diacylglycerol). They are characterised by the distribution of phosphatidylethanolamine (PE) between the inner and outer leaflets. For most membranes, the PE is asymmetrically distributed, with an enrichment of PE in the inner leaflet. But the exosome membrane has a symmetrical PE repartition, and a rapid flip-flop activity between the two leaflets. Paradoxically, exosomes are very rigid and stable at pH=7, with a low lateral diffusion of lipids. However, this rigidity decreases with decreased pH. The presence of sphingomyelin and saturated fatty acids in PE and PC may account for the high rigidity. The presence of tetraspanins, which form microdomains that are not lipid rafts, may explain the pH-dependant loss of rigidity: rigidity of lipid-only vesicles vary only according to the temperature, while pH variations may induce protein plasticity. The physical properties of exosome membranes make them resistant to enzymatic degradation in the circulation. Their symmetrical PE distribution may facilitate their adsorption, but not their fusion with target cells, such as follicular dendritic cells [31]. The mechanisms of cell targeting by exosomes is still a matter of debate. Exosomes contain many adhesion molecules (integrins, ICAM-1, lactadherin) or lysophosphatidylcholine that could promote dendritic cell targeting in vivo [32].

Exosome purification

The biophysical properties of exosomes enable their purification/extraction from culture medium supernatant or physiological fluids (blood [33], ascites [34], malignant effusions [35]). A good manufacturing procedure (GMP) has been described [25]. The size and density of exosomes allow their purification by dialfiltration (500KDa filter) and/or differential ultracentrifugation (100.000g) on D2O/sucrose density cushion or sucrose gradient (1,13 to 1,21 kg/L), which may vary with cell origin. Exosomes can be stored for at least two years at −80°C.

Exosomes purified from serum containing culture medium may be contaminated by serum derived-exosomes [33]. Therefore, clearance of the serum by ultra filtration or ultracentrifugation prior to use in cultures is necessary to remove any preexisting serum vesicles.

Exosomes and the immune system

Immunostimulatory properties of DEX

The first report demonstrating immunostimulatory properties of exosomes derived from APC was performed by Raposo et al who showed that exosomes secreted by EBV-transformed B cells stimulated human CD4+ T cell clones in an antigenic-specific manner [36]. Two years later, we showed that exosomes produced by mouse DC pulsed with tumor peptides induced the rejection of established tumors [4]. These antitumor effects were antigen specific, required T cells and have been associated with long-term survival.

Therefore, the molecular caracterisation and the immunologic properties of DEX were further investigated with in vitro and in vivo experimental models.

First, we developed conditions to obtain optimal peptide loading onto DEX. Exosomal MHC class I molecules could be loaded directly onto DEX with a high peptide concentration after an initial step of acid elution of the exosomal pellet [37]. Exosomal MHC class II molecules could be indirectly loaded by pulsing DC with synthetic peptides [38]. Second, the immunogenicity of exosomal MHC class II molecules was examined. Thery et al, found that H-Y peptide- bearing DEX promoted antigen driven-proliferation of naïve CD4+ T cells in a Marilyn transgenic mouse model [38]. However, H-Y DEX could not directly trigger CD4+ T cell pro-
liferation in vitro unless transferred onto naïve, immature DC. She showed that the I-Ab molecules required for efficient T cell proliferation were presented by DEX and not by the naïve DC. Therefore, it was the first demonstration of functional transfer of MHC molecules from cell to cell in vitro, suggesting that exosomes could be critical cellular components of the immune system.

Third, we confirmed this observation in humans using HLA-A2 DEX pulsed with Mart126-35 peptide. Indeed, human DEX derived from normal volunteers or melanoma patients could promote IFNγ production by Mart1 specific CTL clones only when pulsed onto naïve monocyte derived-DC in vitro. These DEX could also elicit CTL priming from naïve PBL or PBL from melanoma patients in the presence of LPS-activated DC [39]. In HLA-A2 transgenic mice (HHD2), HLA-A2 DEX loaded with Mart1 peptide could induce the differentiation of Tc1 cells in the draining lymph nodes, only if pulsed onto DC prior to the injection into footpad of the mice [39]. Importantly, however, in the MHC class I system, DEX-mediated T cell activation occurred only in the presence of maturing DC or TLR3 and 9 ligands [39, 40]. In this model, T cell priming was as efficient with DEX as with 3.10^5 mature DC (a saturating condition of priming with footpad DC).

Thus, these observations showed that DEX could elicit both CD4 and CD8 specific T cell priming in tumor free mice by transferring functional MHC class I and II/peptide complexes onto recipient maturing DC.

Next, we investigated the immunizing capacities of exosome-based vaccines against established tumors in HHD2 mice. We demonstrated that exosomes mixed with the ligands of TLR3 or TLR9 such as double stranded RNA (Ampligen) or CpG oligodeoxynucleotides (CpG-ODN), were efficient for tumor growth retardation. Using B16F10 co-expressing human HLA-A2.1 and gp100 tumor antigen, we established that DEX mixed with CpG-ODN mediated tumor rejection with an equal or greater efficacy than 3.10^5 A2/gp100 mature DC or 50 μg of gp100 peptide mixed with CpG-ODN respectively [40].

Since breaking tumor induced-tolerance is an important goal to improve current vaccines, we investigated the role of regulatory T cells in preventing DEX efficacy. CD4+CD25+ regulatory T lymphocytes (Tregs) are able to prevent the elicitation of T and NK cell-mediated antitumor immune responses. Tumors induce the expansion of Tregs in humans and mice and prevent the eradication of immunogenic tumors [41, 42]. Conversely, the elimination of Tregs is able to trigger antitumor responses leading to tumor rejection [43–45]. Cyclophosphamide (CTX) has been shown to inhibit Treg functions [46, 47]. Therefore, we studied the role played by Tregs in the inhibition of CD8 response induced by DEX in HHD2 mice bearing a B16A2gp100 tumor [48]. We demonstrated that pre-treatment of tumor bearing-mice with CTX prior to DEX/CpG-ODN vaccines promoted long lasting complete tumor eradication, up to 30%, compared to 10% with DEX/CpG-ODN or CTX alone. Furthermore CpG-ODN were not required for the antitumor effects mediated by DEX in combination with CTX. Moreover, the adoptive transfer of Tregs curtailed the synergistic effects of CTX/DEX. In naïve HHD2 mice, CTX could not promote the capacity of DEX to prime naïve CD8+ T cells, but in Mart1 peptide pre-immunized mice, DEX vaccination after CTX pre-treatment was able to boost the Mart1-induced specific response. Hence, in the presence of CTX, DEX can boost effector T cell functions. Tumor growth was able to promote the expansion of tumor specific T cells since splenic T cells from B16A2/gp100 bearing HHD2 mice produced specific IFNγ in mixed tumor lymphocytes cultures when mice were treated with CTX. Thus, DEX vaccines in association with CTX were able to boost a pre-existing immune response induced by tumor growth or peptides.

Another strategy tested to boost DEX-mediated antitumor activity was to modulate exosome composition to improve intrinsic immunogenicity. Segura et al. showed that the protein composition of exosomes and their biological activity depends on the maturation state of DC [49]. They demonstrated that exosomes secreted by LPS -treated DC (“mature exosomes”) exhibited superior priming abilities than exosomes from untreated DC (“immature exosomes”). Indeed, mature exosomes induced strong antigen specific T cell proliferation both in vitro and in vivo compared to immature exosomes. In male skin graft-bearing female mice, the injection of H-Y peptide loaded mature exosomes triggered effector T cell responses leading to accelerated skin graft rejection. The comparison of mature and immature exosomes [50] revealed their similar
morphology, density and overall protein composition, but they presented a quantitative differences in specific protein composition. MHC class II, CD86 and ICAM-1 molecules were greatly elevated in mature exosomes whereas MFG-E8 and MHC class I molecules were less abundant. The production of exosomes secreted by DC from different KO mice led them to conclude, that expression of ICAM-1 but neither CD86 nor MFG-E8 is necessary on mature exosomes for their increased immunogenicity. CD86 molecules were required on recipient DC to promote T cell activation efficiently. The absence of MFG-E8 on mature exosomes, a molecule that ensures phagocytosis of apoptotic cells by macrophages and DC, did not impair T cell activation [51]. Thus, MFG-E8 is not required for targeting exosomes to recipient DC in mice. However, MFG-E8 can interact with αVβ3 and αVβ5 which are expressed by human DC but not by murine BM-DC. So it is still possible that MFG-E8 might be useful to target exosomes to recipient DC in the human system. In conclusion, exosomes from “in vitro” cultured, mature DC, armed with peptide MHC II complexes and ICAM-1, are efficient in CD4 T cell activation “in vivo”.

The use of tumor-derived exosomes as tumor antigen bearing vehicles

The initiation of a T cell antitumor immune response requires the uptake and processing of tumor antigens by DC and their presentation on MHC I molecules. To load DC with tumor antigens, several “ex vivo” approaches have been tested using tumor lysates, tumor RNA, apoptotic or necrotic debris. Our group has demonstrated by electron microscopy that tumor cells harboured MVBs which release tumor cell-derived exosomes (TEX) to the extracellular medium [15]. We showed that both human and mouse tumor cells constitutively secreted these membrane vesicles, similar to DEX in their morphology, density and expression of certain membrane markers (MHC I, LAMP1, tetraspanins, HSP70-80). Moreover, we reported that TEX contain whole native tumor antigens and could trigger a MHC class I restricted T cell clone reactivity in vitro. TEX represent a source of tumor rejection antigens since they promote T cell-dependent cross-protection against syngenic and allogenic tumors in mice. In cancer patients, malignant effusions like ascitic or pleural effusions accumulate membrane vesicles bearing MHC I, heat shock protein, tetraspanins and tumor antigens (Her2/neu, Mart1, TRP, gp100) indicating similarities with exosomes [35]. Exosomes from melanoma patients carry Mart1 tumor antigen to monocyte-derived dendritic cells (MD-DC) for cross-presentation to Mart1 specific CTL clones. In 7/9 cancer patients, tumor specific lymphocytes could be efficiently expanded from peripheral blood cells using autologous MD-DC pulsed with autologous ascites purified exosomes (Exas). Thus, Exas represent a natural and new source of tumor rejection antigens, opening up a novel immunization strategy for cancer patients. While these findings are encouraging for the development of a future therapy, some data suggest that tumor -derived exosomes have inhibitory effects on the host’s immune system. TEX harbour functional molecules such as FAS-L [52-54], NKG2D ligands [55] or HLA-G [56] involved in the inhibition of effector T cell response. Recently, Liu et al. found that pre-treatment of mice with TEX promoted an accelerated growth of tumors in both immunocompetent and nude mice. The protective effect of TEX pulsed DC on tumors was reduced when mice were pre-treated with TEX. Their results were attributed to a potent inhibition of the cytotoxic activity of NK cells induced by tumor exosomes [57]. In contrast, Dai et al. demonstrated that exosomes secreted by heat-stressed carcinoembryonic antigen-tumor cells exhibited a more potent stimulatory activity onto carcinoembryonic antigen specific CTL than exosomes from non stressed cells in vitro and in vivo [58]. Therefore, as a source of tumor antigens, tumor derived-exosomes should either be pulsed onto ex vivo mature DC or secreted from heat activated cells to mediate immunostimulatory functions in vivo.

Exosomes and clinical immunotherapy

The sophisticated GMP manufacturing process [25] and the optimization of antigen loading [37, 38] together with their immunogenic properties convinced the regulatory authorities to agree to launch clinical trials using DEX in cancer patients. The first phase I clinical trial using autologous DEX was performed at the Gustave Roussy and...
Curie Institute in stage III/IV melanoma patients [59]. DEX were purified from the culture supernatant of day 7 MD-DC of fifteen patients bearing a tumor expressing MAGE3 antigen. MAGE3 peptides (HLA A1/B35) were loaded onto MD-DC (in the first six patients) or directly onto DEX (in nine patients). MAGE3 class II peptides (DP04) or tetanus toxin class II epitopes were also loaded onto MD-DC to confer a helper effect. Two dose levels of cryopreserved DEX (0.13 versus 0.40.10^{14} MHC class II molecules) or peptides (10 versus 100 μg/ml) were tested by weekly sc/id injection for 4 weeks, followed by vaccinations every 3 weeks in patients who achieved objective tumor response. GMP DEX were successfully prepared from all patients in the study. The therapy schedule was well tolerated with toxicities under grade II. Although the aim of a phase-I clinical trial is not designed to conclude that the treatment has a significant activity, some clinical benefits were observed (one partial response, one minor response, one mixed response and two stable diseases) in skin and lymph node target lesions. The T cell immunomonitoring did not reveal any significant anti MAGE3 A1/B35 CTL expansion nor clinically significant delayed type hypersensitivity (DTH) responses. Unexpectedly, the number of circulating CD3-/CD56+ NK cells/mm^3 significantly increased after three vaccinations with exosomes. More than 50% of patients exhibited enhanced NK cell cytotoxic capacity and IFNγ secretion following an ex vivo boost with IL-2 or DC. This first Phase I trial together with a second one [60] in non small cell lung cancers (NSCLC) established the feasibility and safety of DC-derived exosomes-based vaccination in metastatic patients.

Based on these encouraging results, a Phase II/III trial using DEX in NSCLC has been designed as outlined in Fig. 1. In parallel, the group of Adams has launched a Phase I/II trial in
ovarian cancer patients aimed at assessing the association of chemotherapy/immunotherapy with a TLR3 agonist followed by a vaccination with tumor cell-derived exosomes isolated from autologous ascitic fluids [personal communication and 61] as outlined in Fig. 2.

Other potential therapeutic applications of exosomes

Aline et al. established that Toxoplasma gondii antigens pulsed DC-derived exosomes could prime an antigen specific cellular and humoral immune response that provides protection against both acute and chronic toxoplasmosis in mice [62]. This was the first demonstration that DEX can be used for immunoprophylaxis against pathogens.

Recently, Robbins et al. demonstrated in two studies that exosomes produced by IL10 treated BM-DC or by genetically modified BM-DC expressing FAS-L could be immunosuppressive in vivo [63, 64]. These tolerogenic exosomes are able to suppress inflammation in a murine footpad model of DTH and to reduce the severity of an established collagen-induced arthritis. These effects were antigen-specific and required the presence of MHC class II on exosomes as well as the integrity of the vesicles. Taken together, these data suggest that immature or genetically modified DC confer immunosuppressive activity to the secreted exosomes. It is therefore conceivable that DEX might be useful in controlling autoimmune diseases.
Conclusion

Exosomes are produced by numerous cell types and can be defined by three major criteria: a size of 60–100 nm in diameter, a density of 1.13 to 1.21 g/dL in a sucrose gradient and an endocytic origin (enrichment in HSP 70, tetraspanins, Tsg101, Alix, MHC molecules). For immunologists, exosomes represent a cell free vaccine which may be a little bit easier to define, store and manipulate compared to a cell-based vaccine. To date, the physiological role of the different exosomes produced in vivo is not understood and remains difficult to evaluate. The in vivo stimulatory properties of DEX have been demonstrated in preclinical studies using DEX or TEX. It is established that ICAM-1 is necessary for their immunogenicity and that the use of adjuvants or the depletion of Tregs by cyclophosphamide strongly enhanced antitumor effects. The feasibility and the safety of DEX assessed in phase I clinical trials are encouraging for the future development of exosomes in cancer immunotherapy.

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