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Extracellular vesicles in cancer — implications for future improvements in cancer care

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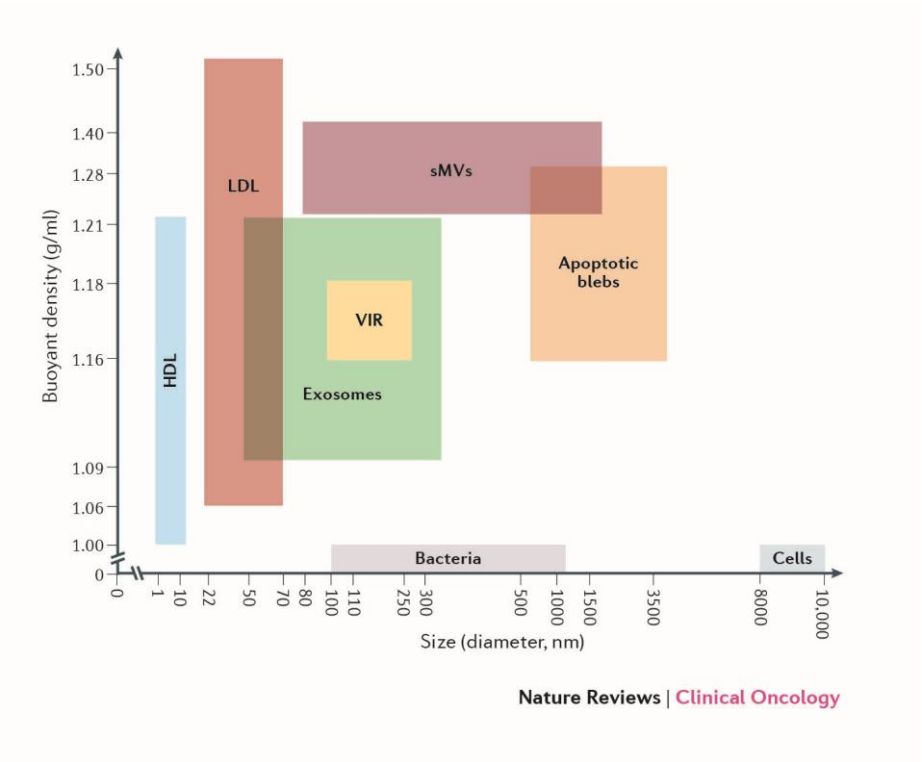

Supplementary Figure 1 | **Physical properties and characteristics of extracellular vesicles.** The major classes of extracellular vesicles (EVs), including exosomes and shed microvesicles (sMVs), are heterogeneous in diameter and buoyant density. Other entities co-isolated with EVs include lipoprotein particles (light, low-density lipoprotein (LDL); heavy, high-density lipoprotein (HDL)), viral particles (VIR), apoptotic blebs, bacteria, and cells. As lipids have a density of ~1 g/cm, and proteins and RNA >1.3 g/cm, density gradients can be used to separate subpopulations of EVs with differing ratios of lipids, RNAs, and proteins from other entities. Density gradients can be used to purify and isolate EV classes from soluble proteins, free RNA, and protein–RNA complexes. In addition to particle diameter and buoyant density, other physical parameters of EVs, such as light scatter — which is correlated not only with size, but also with geometry and composition — can be measured using flow cytometry.

Supplementary Box 1 | **Clinically relevant approaches for EV isolation**

The demands of clinical applications involving diagnostics and therapeutics, such as low costs, reliability, and speed, can eventually be met with modifications to existing technologies for improved scalability. Over the past decade there has been much interest in blood-derived extracellular vesicles (EVs) because they contain clinically relevant information — in the form of oncoproteins, RNA (mRNA, microRNA (miRNA), long noncoding RNA (lncRNA), and fusion gene mRNA), and lipids (see **BOX 2** and **TABLE 2**). However, the isolation of EVs from blood and urine is a challenge due to the presence of bulk proteins and lipoproteins, which undoubtedly will attenuate intrinsic EV protein and/or RNA signatures. This problem is exacerbated by the lack of knowledge of the percentage of disease-derived EVs in the total blood EV pool, which is critical for evaluating biomarkers: at this juncture in time, there is no robust enumeration technique for determining specific EV concentrations in blood or for comparing and standardizing EV purity that would enable inter-laboratory comparisons. Isolation of EVs from blood (plasma or serum) by differential centrifugation, density (sucrose, percoll, or iodixanol) gradient centrifugation, and precipitation methods (for example, synthetic polymers such as polyethylene glycol (PEG), acid precipitation, and others) have serious shortcomings in that they result in co-isolation of protein aggregates, in particular, protein AGO2–RNA complexes[S1] and high-density lipoprotein (HDL) particles, which attenuate EV cargo signals and confound interpretation of miRNA profile, respectively. HDL particles have been reported to contain bound endogenous miRNAs, indicating that HDL might have a role in cell–cell communication by horizontal transfer of miRNAs[S2]. Recently, it was shown that EVs can be rapidly isolated from biological fluids, such as plasma, using simple ready-made size-exclusion chromatography (SEC) columns[S3–S6]. This one-step isolation procedure for plasma EVs is highly reproducible and an effective means of eliminating >95% of extraneous protein from plasma. Other emerging methods that are scalable include field-flow fractionation[S7], sequential low-g force centrifugal ultrafiltration[S8], and free-flow electrophoresis (G. Weber, personal communication).

Generation of EVs for therapeutic studies.

By virtue of their bioactive cargo (see **TABLE 1**) EVs have inherent therapeutic potential[S9–S11]. For example, exosomes secreted by human mesenchymal stem cells (MSC) have been used in tissue regenerative medicine to reduce infarction size in a mouse model of myocardial

ischaemia–re-perfusion injury[S12]. For these studies, large-scale production of functional homogeneous MSC-derived exosomes was accomplished using SEC-based high-performance liquid chromatography (HPLC) fractionation. In another therapeutic application, exosomes from dendritic cells (and tumour cells) have been trialed in cancer vaccine studies[S13–S16] (see **BOX 3**). Navabi and colleagues[S17] described a large-scale production method combining ultrafiltration and sucrose–deuterium oxide (UC cushion) for generating good manufacturing (GMP)-grade exosomes from ascites fluid of patients with ovarian cancer for use in clinical trials.

Supplementary Box 2 | **Paget's 'seed and soil' hypothesis — a basic tenet of metastasis**

Sir Stephen Paget and Dr James Ewing pioneered metastasis research in the late 19th and early 20th centuries by proposing two major theories to explain the organ specificity of metastasis. In 1889, Paget[S18] proposed that sites of secondary cancer growth are not a matter of chance, but rather that some organs provide a more 'fertile' environment than others for metastatic growth. This concept — the 'seed and soil' hypothesis — was at odds with James Ewing's extant theory at the time, which posited that metastatic dissemination patterns can be solely accounted for by vascular connections to the primary tumour[S19]. Ewing's viewpoint prevailed for several decades and the 'seed and soil' hypothesis languished in the shadows for many years. Not until the 1980s did the seed and soil hypothesis re-gain traction, following the observation by Isaiah Fidler[S20], using radiolabelled cancer cells, that although circulating cancer cells equally distribute to all tissues, metastases developed only in selected organs (that is, organotypic metastases). Since then, organotypic metastasis has been a cornerstone in metastasis research, with the focus turning to unravelling the molecular mechanisms that interlink 'seed and soil' to promote metastases.

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