**Supplementary METHODS**

***Tumor Tissue Analyses***

Archival FFPE specimens of patients’ primary and metastatic tumors (minimum tumor content 20%) obtained from routine diagnostic and/or therapeutic procedures were tested for *BRAF*V600, *KRAS*G12/G13, and *EGFR*exon19del/L858Rmutations in MD Anderson’s CLIA-certifiedMolecular Diagnostics Laboratory. DNA was extracted from microdissected paraffin-embedded tumor sections and analyzed with a PCR-based DNA sequencing method that used primers designed by MD Anderson’s Molecular Diagnostic Laboratory. In January 2011, the assay was changed to mass spectrometric detection (MassARRAY, Sequenom, San Diego, CA), and in March 2012, to next-generation sequencing (Ion Torrent, Life Technologies, Carlsbad, CA). The lower limit of detection for these technologies is approximately 5-10% MAF and is influenced by clonal heterogeneity and the presence of normal tissue.

***Exosome Depleted Plasma***

Plasma samples were pre-filtered with 0.8 µm filter, diluted 2:1 (30%) with PBS and ultra-centrifuged at 150,000 x g for 4 hours. The supernatant was carefully transferred into a new tube and considered exosome depleted. This procedure depletes about 75% of extracellular vesicles and is described in detail in Eitan et al. 2015 (PMID 25819213).

***Protein Isolation***

Exosomes and cfDNA from plasma samples pre-filtered with 0.8 µm were bound to ExoLution™ Plus isolation columns and washed according to the manufacturer’s instructions. Subsequently, the columns were moved to a new collection tube, 400 µL of lysis buffer (M-PER, Thermo Fisher Scientific 78501) was added to the column and incubated for an hour at 4°C degrees. The protein lysate was eluted from the columns by centrifugation for 5 min at 5000 x g.

***Western Blot***

The exosome protein lysates were spun for 20 min at 8,000 x g to remove membrane debris. The supernatant was transferred into a new tube and incubated with protein G beads to deplete endogenous IgG antibodies. The tubes were placed on a magnet and the supernatant was transferred into a new tube. 30 µL of the protein sample was added to 10 µL of 4X sample buffer (NuPage LDS Thermo Fisher Scientific NP0007) and centrifuged for 5 mins at 8000 x g. The supernatant was transferred into a new tube and placed for 10 minutes in 80°C degrees and then placed for 2 minutes on ice. 10µg of U87 cells protein lysate were added to sample buffer and were used as positive control. The entire sample (40µl ) was loaded to a 4-12% Bis-Tris gel (Invitrogen NP0322BOX) and run with MOPS SDS running buffer (Thermo Fisher Scientific NP0001). The sample was then transferred to a nitrocellulose membrane and blotted with the following antibodies from Abcam: FLOT1 (ab133497 1:2000 dilution), TSG101 (ab83 1:200 dilution) and calnexin (ab22595 1:1000 dilution). The following secondary antibodies were used: Goat anti-Mouse IgG HRP (Thermo Fisher Scientific PI-31430) and Goat Anti-Rabbit IgG HRP (Thermo Fisher Scientific PI-31460) antibodies. Three samples were exposed only to secondary antibodies (Anti-Mice and Anti-Rabbit) to control for nonspecific signal. The western was developed with WESTERN LIGHTNING PLUS-ECL (Thermo Fisher Scientific 50-904-9323)The signal was detected using LI-COR Biosciences instrument.

***Nanoparticle Tracking Analysis (NTA)***

Intact vesicles from 2 mL plasma was eluted from an ExoLution ™ column into 400 µL 1x Elution Buffer using a 5 min incubation step followed by a 5 min spin at 500 x g to collect the EVs for analysis with a NanoSight LM10 instrument. Briefly, the sample was diluted 1:100 in elution buffer, loaded into the assembled sample chamber and brought into focus using the thumbprint region as a reference; three 30-s video images were acquired and analyzed using the NanoSight NTA 2.3 software according to the manufacturer’s instructions.

***Scanning Electron Microscopy (SEM)***

Intact vesicles were eluted from ExoLution ™ columns into 280 µL 1x Elution Buffer using a 5 min incubation step followed by a 5 min spin at 500 x g to collect the eluted vesicles for subsequent cryo-fixation. Undiluted sample was applied to a glass microscopy slide with a cover slip and snap frozen in liquid nitrogen. After removal of the cover slip the frozen specimen was fixed with 2.5% glutaraldehyde in exosome elution buffer, washed once with elution buffer and once with water, then dehydrated using acetone. The acetone was removed using critical point drying with liquid carbon dioxide. Mounting was performed on aluminum stubs with tempfix adhesive and contacting using colloidal silver. Samples were examined with a Zeiss Auriga Workstation after sputter-coating with 3-5 nm platinum.

**Supplementary Table 1.** Sensitivity and specificity for *BRAF*, *KRAS*, and *EGFR*mutations testing of plasma exosomal nucleic acids and cell-free DNA (exoNA) using the next-generation sequencing and plasma cell-free DNA using droplet digital PCR or BEAMing digital PCR.

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| --- | --- | --- | --- |
| **Method** | **Mutations** | **Sensitivity % (95% confidence interval)** | **Specificity % (95% confidence interval)** |
| **NGS of exoNA** | ***BRAF*V600** | 95% (75%-100%) | 100% (85%-100%) |
| ***KRAS*G12/G13** | 100% (80%-100%) | 100% (87%-100%) |
| ***EGFR*exon19del/L858R** | 75% (19%-99%) | 100% (91%-100%) |
| **Droplet digital PCR of cfDNA** | ***BRAF*V600** | 89% (67%-99%) | 100% (85%-100%) |
| ***KRAS*G12/G13** | 95% (80%-100%) | 96% (79%-100%) |
| ***EGFR*exon19del/L858R** | 67% (9%-99%) | 100% (90%-100%) |
| **BEAMing digital PCR of cfDNA** | ***BRAF*V600** | 93% (66%-100%) | 96% (78%-100%) |
| ***KRAS*G12/G13** | 100% (80%-100%) | 85% (62%-97%) |
| ***EGFR*exon19del/L858R** | 100% (40%-100%) | 100% (88%-100%) |

**Supplementary FIGURE LEGEND**

**Supplementary Fig. 1. Isolation of exosomes by spin-column purification (A)** Exosomes bound to the ExoLution™ column were lysed and analyzed by Western Blot for the exosomal markers FLOT1 and TSG101 and the cellular marker Calnexin. Samples were isolated from 0.5, 1 and 2 mL of two plasma pools and from exosome-depleted plasma as an internal control **(B)**. Protein extracts from 2 mL of plasma pool #1 and 2 mL of exosome-depleted plasma pool #2 were run alongside the samples presented in A but exposed only to mice and rabbit secondary antibodies to reveal a single unspecific band at 150 kDa. **(C)** Scanning electron microscopy using 8190x magnification show plasma vesicles eluted from the ExoLution™ column. The scale bar depicts 1 µm and white arrows point out 50-200 nm structures identified as exosomes or other extracellular vesicles. **(D)** Representative nanoparticle tracking analysis of plasma vesicles eluted from an ExoLution™ column (NanoSight LM10). Red bars indicate +/- 1 standard error of mean from three measurements of the same sample, resulting in the above values for particle count and maximum peak size. Using eluted vesicles from 4 independent plasma samples, we detected an average of 6.0 x 10^10 +/- 16% particles per mL with an average maximum peak size (mode) of 180.6 +/- 7% nm.

**Supplementary Fig. 2.** The median survival time of 25 patients with RMH scores of 0 or 1 (dashed blue line; 8.4 months; 95% CI, 6.0–10.8 months) was significantly longer than that of 18 patients with RMH scores of 2 or 3 (red line; 6.0 months; 95% CI, 2.3–9.7 months; *P*=0.017).