Supplementary Table 1 for Figure 1. Characterization of neuroblastoma cell lines

Phenotypical characterization of the different neuroblastoma cell lines used in this study, including phenotype and GD2 expression levels. GD2 surface expression was established by flow cytometry (**Supplementary Figure 2**).

Supplementary Figure 1. Multimodal treatment protocol for high-risk neuroblastoma patients

Scheme showing the different phases (induction, consolidation and maintenance) of the treatment for high-risk neuroblastoma patients. The immunotherapy regimen, as approved in Northern America, is subdivided in alternating cycles of dinutuximab combined with GM-CSF or IL-2, and isotretinoin (13-cis-retinoic acid). Each cycle consists of 28 days. Figure adapted from Smith, V.; Foster, J. High-Risk Neuroblastoma Treatment Review.[1] ASCT, autologous stem cell transplantation.

Supplementary Figure 2 for Figure 1. Binding capacity of dinutuximab to GD2 on neuroblastoma cell lines and ADCC of GD2-negative neuroblastoma cell lines

(A, B, C) Binding capacity of increasing concentrations of dinutuximab (dimab) to GD2 (expressed as MFI) as measured by flow cytometry on GD2-positive cell lines (A) NMB (light grey triangles), IMR-32 (black squares) and LAN-1 (dark grey circles) and on cell lines expressing lower or none GD2 (B) SHEP-2 (light grey triangles), SK-N-AS (dark grey circles), SH-SY5Y (dark grey diamonds) and SK-N-BE (black squares), and on the GD2-positive primary patient-derived 691B cell line (light grey diamonds) (C). NMB, IMR-32 and LAN-1 n=1, of 2 individual experiments. SHEP-1, SK-N-AS, SH-SY5Y and SK-N-BE n=2, of 2 individual experiments. 691B n=3, of 3 individual experiments. **(D)** ADCC of GD2-negative cell lines SH-SY5Y and SK-N-BE opsonized with (+) or without (-) dinutuximab (dimab) by *in vitro* stimulated neutrophils with GM-CSF (light grey bars) or G-CSF (dark grey bars). SH-SY5Y and SK-N-BE n=2, of 1 individual experiment. Statistical differences were tested with unpaired T-test used to test statistical differences.

Supplementary Figure 3 for Figure 1. Area under the curve for statistics of Figure 1B-D

Areas under the curve (AUC) of ADCCs of NMB, IMR-32 and LAN-1 cells with dinutuximab titration **(A)**, with increasing T:E ratios **(B)**, or with cytokines titration **(C)**, by neutrophils stimulated *in vitro* with GM-CSF (light grey bars) or G-CSF (dark grey bars). NMB n=4, IMR-32 n=4-6, LAN-1 n=4-6, of 3 individual experiments. Statistical significance was tested with unpaired T-test to test the difference of the AUC.

Supplementary Figure 4 for Figure 4. Effect of G-CSF treatment on proliferation rate and GD2 expression

(A-B) Representative flow cytometry plots of intracellular total STAT3 plotted against intracellular pSTAT3 (A) and pSTAT3 intracellular expression levels relative to total STAT3 in IMR-32 cell line and neutrophils over the course of G-CSF exposure (0 to 20 minutes). N=2, of 2 individual experiments (B). **(C)** GD2 expression (expressed as MFI) measured over time on IMR-32 cells cultured in absence (control) or presence of G-CSF for 7, 14 and 21 days detected with 1 µg/mL of dinutuximab. N=3-13, of 6 individual experiments. Statistical significances were tested with ordinary one-way ANOVA with post-hoc Sidak test. **(D)** GD2 expression (expressed as MFI) measured over time on 691B cells cultured in absence (control) or presence of G-CSF for 7, 14 and 21 days detected with 2.5 µg/mL of dinutuximab. N=4-14, of 6 individual experiments. Statistical differences were assessed with ordinary one-way ANOVA with post-hoc Sidak test. **(E-F)** G-CSF receptor expression (expressed as MFI) measured over time on IMR-32 and 691B cells cultured in absence (control) or presence of G-CSF for 7, 14 and 21 days. IMR-32 n=2-3, 691B: n= 3-4, of 3 and 4 individual experiments, respectively. Statistical significance was assessed with ordinary one-way ANOVA with post-hoc Sidak test. **(G-H)** Area under the curve (AUC) of one-week proliferation rates of IMR-32 cultures (G) and 691B cultures (H) treated without (white bars) or with G-CSF (dark grey bars). IMR-32 n=4, 691B n=3, of 3 and 4 individual experiments, respectively. Statistical significance was tested with unpaired T-test to test the difference of the AUC. **(I)** Population doubling time (PDT, expressed in days) of IMR-

32 cells upon being cultured in the absence (control, white symbols) or presence of G-CSF for 7 (circles), 14 (triangle) and 21 (squares) days. N=1-6, of 6 individual experiments. Statistical significances were tested with ordinary one-way ANOVA with post-hoc Sidak test. **(J)** PDT (expressed in days) of patient-derived 691B cells upon being cultured in the absence (control, white symbols) or presence of G-CSF for 7 (circles) days. N=3, of 3 individual experiments. Statistical significance was assessed with paired T-test.

Supplementary Table 2 for Figure 5. GM-CSF injected patient characteristics

GM-CSF injected patient characteristics, including disease stage as determined by the INRG, age at time of blood sampling (months), gender and treatment cycle 1-5.

Supplementary Figure 5 for Figure 5. *In vivo* GM-CSF stimulated neutrophils show enhanced *ex vivo* killing of IMR-32 neuroblastoma cells

ADCC of IMR-32 cells opsonized with (+) or without (-) dinutuximab (dimab) by control neutrophils from healthy donors (white bars) or by neutrophils from neuroblastoma patients that were injected with GM-CSF (striped light grey bars). N=10 healthy donors, n=8 patients, of 4 individual experiments. Statistical differences were tested with ordinary one-way ANOVA with post-hoc Sidak test.