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Supplemental Information

Development and Preliminary Clinical Activity of PD-1-Guided CTLA-4 Blocking Bispecific DART Molecule

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Supplementary Information

Table S1. Comparative ligand blocking activity of MGD019, related to Figure 2.

Assay, N≥3	Ipilimumab*, EC_{50}	Nivolumab*, EC_{50}	MGD019, EC_{50}
PD-1 blockade (PD-L1 binding to Jurkat/PD-1 cells)	N/A	0.274 nM	0.416 nM
CTLA-4 blockade (B7-1 binding to Jurkat/CTLA-4 cells)	1.35 nM	N/A	4.81 nM
CTLA-4 blockade (B7-1 binding to Jurkat/PD-1+CTLA-4 cells)			0.014 nM

* - replicas of ipilimumab and nivolumab created at MacroGenics based on available sequence.

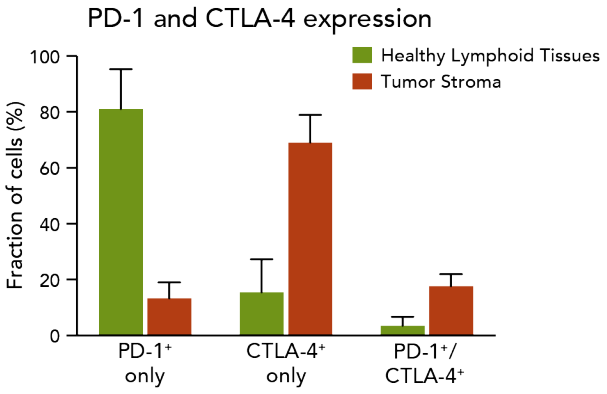

Figure S1. PD-1 and CTLA-4 co-expressed by TILs, related to Figure 1. Relative frequency of cells expressing PD-1 mRNA, CTLA-4 mRNA or both in stromal area of representative cores (N=12) of breast, colorectal or lung cancer or lymphoid tissues from healthy donors (N=7) detected by ISH and quantified with HALO software. Mean and SEM are depicted.

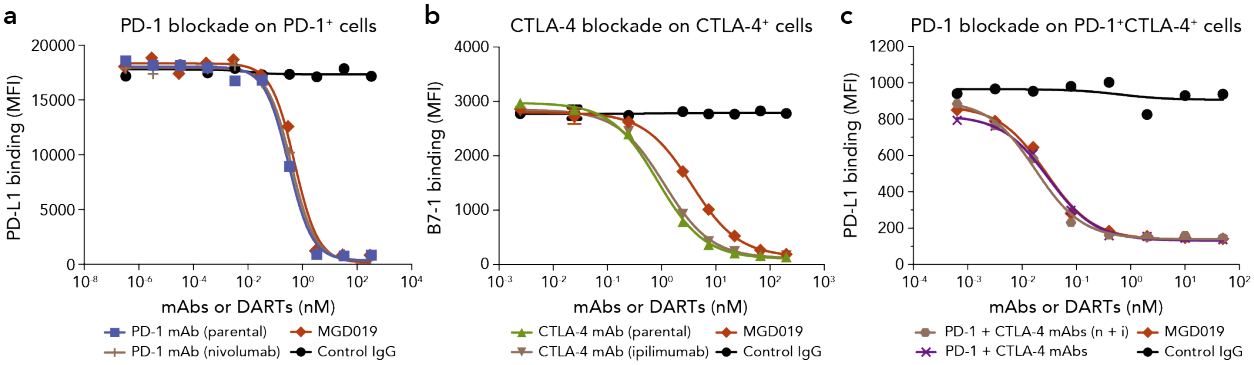

Figure S2: MGD019 blocks PD-1 and CTLA-4, related to Figure 2. a, MGD019 (red diamonds), retifanlimab (blue squares), nivolumab replica (tan lines) or isotype control (black circles) prevents binding of PD-L1 to Jurkat/PD-1 cells. **b,** MGD019 (red diamonds), parental CTLA-4 mAb (green triangles), ipilimumab replica (tan triangles) or isotype control (black circles) prevents binding of B7-1 to Jurkat/CTLA-4 cells. **c,** MGD019 (red diamonds), a combination of its parental antibodies (purple crosses), a combination of nivolumab (n) plus ipilimumab (i) (tan hexagons) and isotype control (black circles) prevents binding of PD-L1 to Jurkat/PD-1+CTLA-4 cells.

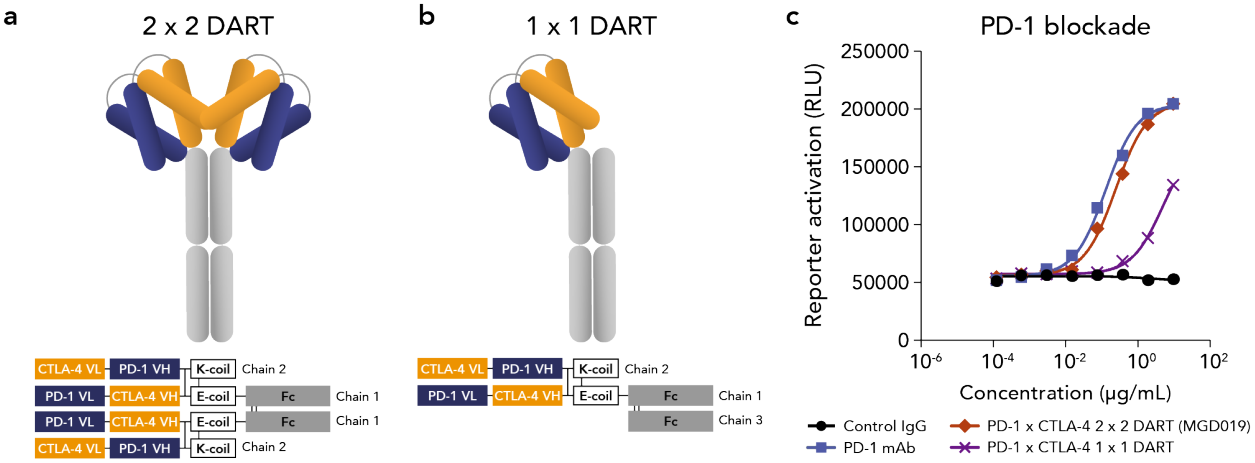

Figure S3: Molecular format influences potency of PD-1 x CTLA-4 bispecific inhibitor in vitro, related to Figure 2. a, Schematic representation of tetravalent bispecific (2 x 2) and **b,** bivalent bispecific (1 x 1) Fc-bearing DART molecules and their molecular design. **c,** rescue of cell signaling detected with PD-1/Luc^{NFAT} reporter systems in the presence of 2 x 2 DART molecule (red diamonds), 1 x 1 DART molecule (purple crosses), parental PD-1 mAb (blue squares) or isotype control (black circles).

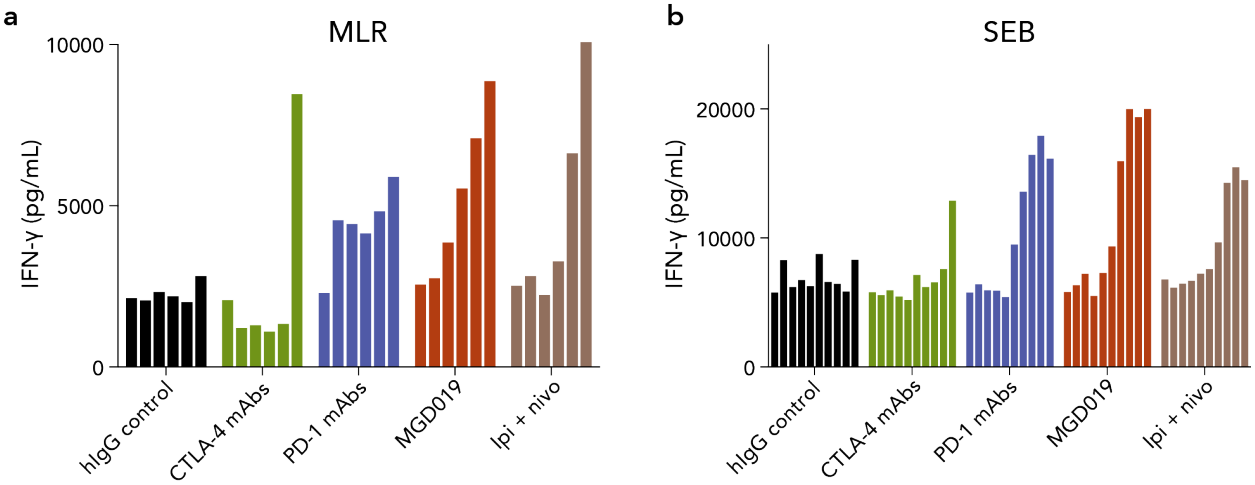

Figure S4: MGD019 enhances T cells activation in vitro equipotent to combination of ipilimumab and nivolumab, related to Figure 3. a, Representative MLR and **b,** SEB restimulation assays out of least 10 repeats are shown. Mean interferon gamma concentrations in the supernatants are depicted.

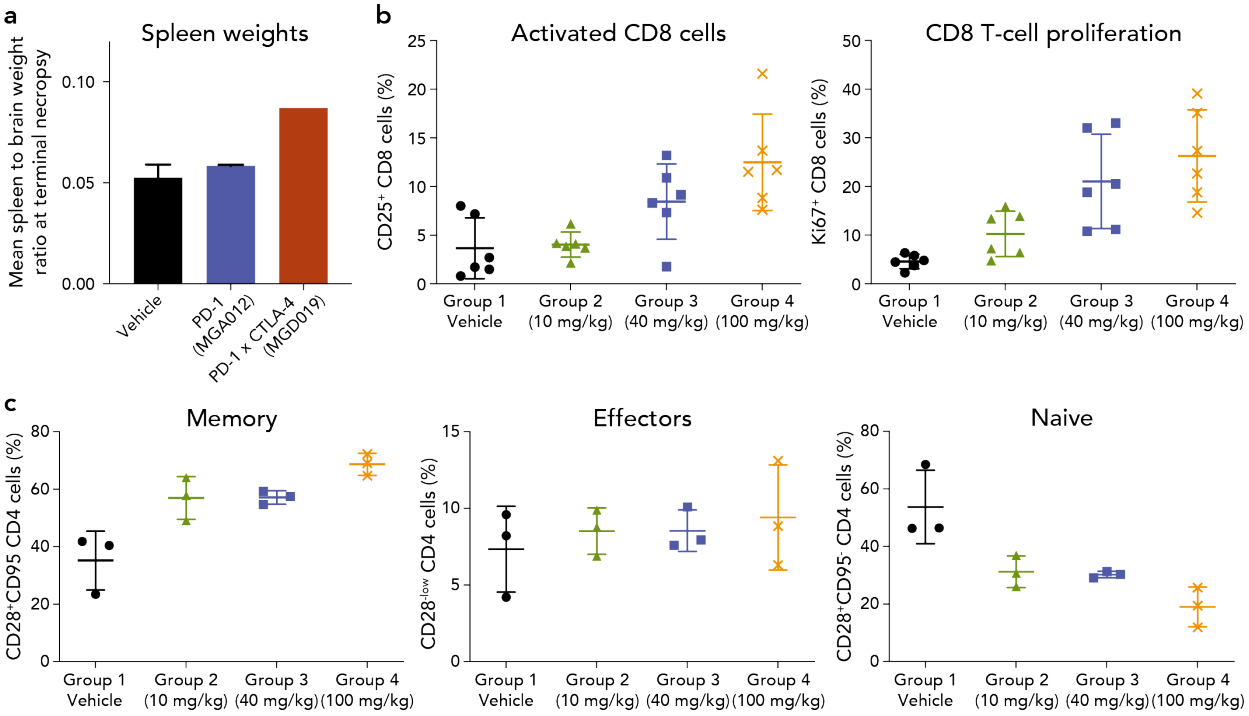

Figure S5: MGD019 supports T-cell expansion in vivo, related to Figure 4. a, Cynomolgus monkey (3F/3M) were infused with 100 mg/kg/dose MGD019 at Day 1, 8, 15, and 22; in a separate study, animals received weekly IV administrations of 150 mg/kg parental PD-1 mAb (2M/2F). Spleen weights at terminal necropsy were calculated as fraction of brain weight. **b,** Splenocytes from MGD019-treated cynomolgus monkeys (N=6 per group) were isolated and analyzed for expression of CD25 and Ki67 by CD8+CD3+CD45+ cells. **c,** Cynomolgus monkey splenocytes were processed as described in previous and analyzed for CD28/CD95 co-expression in CD4+ T cells as in Fig 4. Fractions of cells expressing CD28 and CD95 (memory), CD95 with low CD28 expression (effectors) or CD28 with low CD95 (naïve) are shown. Mean and SEM are depicted.

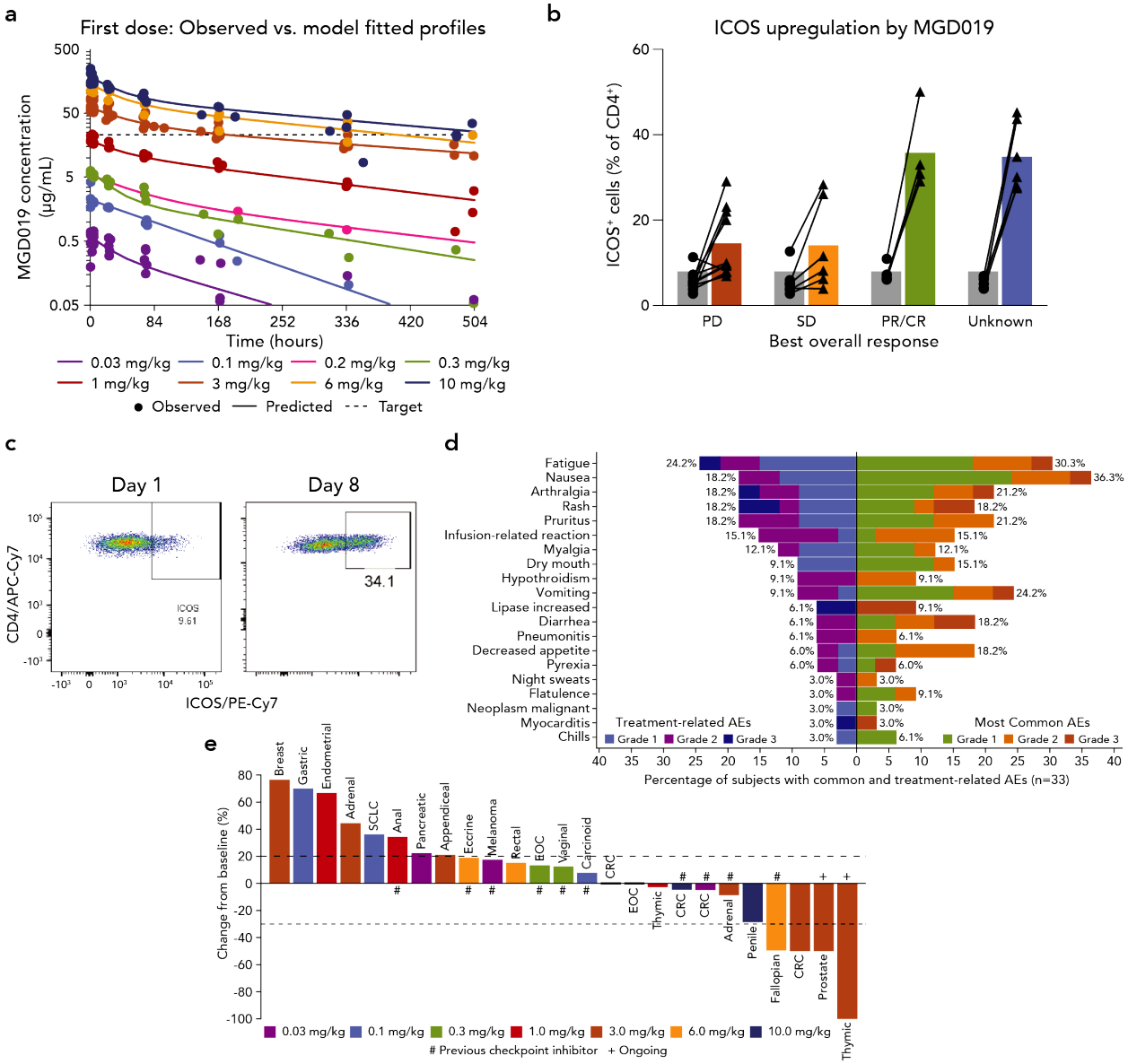

Figure S6: MGD019 demonstrates evidences of dual checkpoint blockade and efficacy in patients related to Figure 5. a, First-dose PK profiles of 0.03 to 10 mg/kg. Symbols and solid lines represent observed data and model fitted median curves, respectively. **b,** Upregulation of ICOS expression (between day 1 and day 8) by circulating $CD4^+$ T cells in patients treated with MGD019 grouped by best overall response (N=28). Bars depict mean values. **c,** Representative flow cytometry images from a patient treated with 3 mg/kg of MGD019. Gated on live CD3+CD4+ cells. **d,** Tornado plot indicating most common treatment-related adverse events (TRAEs) and corresponding incidence of these adverse events irrespective of attribution, color coded by CTCAE v5 severity scoring. **e,** Waterfall plot of response-evaluable population in MGD019 dose escalation (n=25). Bars represent best percent change from baseline in target lesion tumor burden.

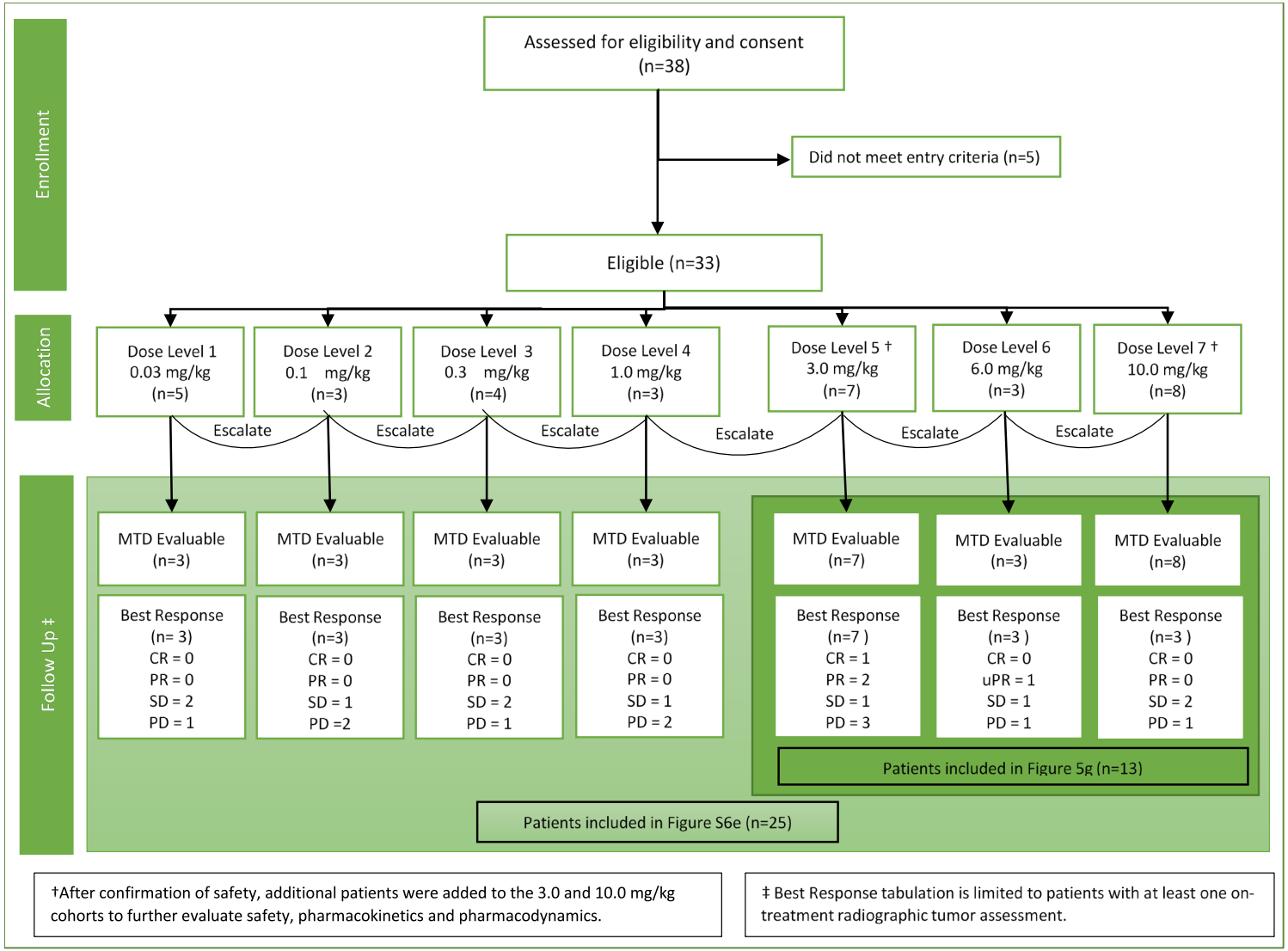

Figure S7: Design and preliminary activity of First-In-Human study of MGD019, Related to "MGD019 Phase 1 Clinical Study" in the STAR Methods section.