| # | Age | Gender | Histology | Subtype (Verhaak) | Tumor Initiation | Differentiation | LDA |
|---|---|---|---|---|---|---|---|
| #1 | 68 | M | GBM | Mes | + | + | 8 |
| #2 | 25 | M | GBM | Mes | + | + | 6 |
| #3 | 67 | M | GBM | Mes | n.d. | + | n.d. |
| #4 | 76 | F | GBM | Mes | + | + | 6 |
| #5 | 66 | M | GBM | Proneural | + | + | 6 |
| #6 | 68 | M | GBM | Mes | + | + | 1.6 |
| #7 | 49 | M | GBM | Mes | + | + | 1.3 |
| #8 | 79 | M | GBM | Classic | + | + | 6 |
| #9 | 68 | F | GBM | Classic | + | + | 6 |
| #10 | N/A | M | GBM | Classic | + | + | 2.6 |
| #11 | 67 | M | GBM | Proneural | + | + | 4 |
| #12 | 59 | M | GBM | Neural | + | + | 3 |
| #13 | 73 | F | GBM | Proneural | + | + | 2.7 |
| #14 | 70 | F | GBM | Mes | + | + | 2.4 |
| #15 | 72 | M | GBM | Classic | + | + | 6 |
| #16 | 65 | F | GBM | Proneural | + | + | 2.7 |

**Table S1. GSC characterisation**
LDA (limiting dilution assay) was measured as detailed in the method section. Differentiation was induced by switching cells from mitogen-containing media to DMEM supplemented with 10% FBS. Differentiation was considered as positive according to morphological changes of adherent cells within 10 days. Tumour initiation was assessed by intracranial implantation in nude mice and considered as positive when at least half of animals develop tumours within 3 months. n.d.: not determined.

| Target | Left Primer | Right Primer |
|---|---|---|
| ACTB | GGACTTCGAGCAAGAGATGG | AGCACTGTGTTGGCGTACAG |
| ADM | CGTCGGAGTTTCGAAAGAAG | CCCTGGAAGTTGTTCATGCT |
| APLN | GGAAGTGCAGCAGGAATAGC | ACACACAAAGTTGGGCATCA |
| APLNR | GAGTGCTGGGAAGGACTCTG | ACTGGTTGTCTGCCCCATAG |
| CSTB | CCAGGGAGCAAGACAGAGAC | GAGACTGGCGTTCTCCAAAG |
| CST3 | ACCAGCCACATCTGAAAAGG | AGAGGGGACAATCAGTGTGG |
| CTGF | GGAAAAGATTCCCACCCAAT | TGCTCCTAAAGCCACACCTT |
| EDIL3 | CCCGAGGATTTAATGGGATT | GTGGGCCTGAGCATTTGTAT |
| EFEMP1 | CAGGACACCGAAGAAACCAT | GTTTCCTGCTGAGGCTGTTC |
| FN1 | ACCAACCTACGGATGACTCG | GCTCATCATCTGGCCATTTT |
| FSTL1 | GCACAGGCAACTGTGAGAAA | CATAGTGTCCAAGGGCTGGT |
| HSPG2 | CTGCCGTAATCTCCACCAAT | CTTTTGGCTGTGCAGATGAA |
| IGFBP7 | AAGTAACTGGCTGGGTGCTG | TATAGCTCGGCACCTTCACC |
| LAMA5 | TGACCTTTTCTGGCTCGTCT | GTTCAGCACAAAGGGCTCTC |
| LGALS1 | CTCTCGGGTGGAGTCTTCTG | ACGAAGCTCTTAGCGTCAGG |
| LGALS3BP | ACCAATGAAACCAGGAGCAC | GCATCCACACTCATGGTGAC |
| MIF | GTTCCTCTCCGAGCTCACC | TGCTGTAGGAGCGGTTCTG |
| NES | AACAGCGACGGAGGTCTCTA | TTCTCTTGTCCCGCAGACTT |
| PRSS23 | ACTTACGAAGAGGCCAAGCA | GTCCTTCCCAAAAATGCTGA |
| PTX3 | GTGGGTGGAGAGGAGAACAA | TTCCTCCCTCAGGAACAATG |
| SERPINE1 | CTCTCTCTGCCCTCACCAAC | GTGGAGAGGCTCTTGGTCTG |
| SOX2 | ACACCAATCCCATCCACACT | GCAAACTTCCTGCAAAGCTC |
| SRGN | CAGGGTTTGAGGTTTTGGAA | CCGCGTAGGATAACCTTGAA |
| TGFB2 | TGCTTTGGCTTTCTGGTTCT | TTTGTTTGTGGTGCAGTGGT |
| THBS1 | AGGCATGTTCCAGTTTCACC | GCTGGCACCACCTTTATTGT |
| TIMP1 | AATTCCGACCTCGTCATCAG | TGCAGTTTTCCAGCAATGAG |

**Table S2. RT-PCR primers**

| | | Peptidome | | Proteome | |
|---|---|---|---|---|---|
| Gene | Protein name | Score | emPAI | Score | emPAI |
| | Cytokines | | | | |
| *ADM* | Adrenomedullin | 483 | 24.02 | - | - |
| *APLN* | Apelin | 170 | 42.17 | - | - |
| *CTGF* | Connective tissue growth factor | 34 | 0.16 | - | - |
| *FSTL1* | Follistatin-related protein 1 | 55 | 0.17 | 36 | 0.17 |
| *PTX3* | Pentraxtin-related protein 3 | 86 | 0.51 | 124 | 0.91 |
| *IGFBP7* | Insulin-like growth factor-binding protein 7 | - | - | 67 | 0.44 |
| *MIF* | Macrophage migration inhibitory factor | - | - | 50 | 0.53 |
| *TGFB2* | Transforming growth factor beta-2 | - | - | 46 | 0.12 |
| *LGALS1* | Galectin 1 | - | - | 49 | 0.43 |
| | Proteases | | | | |
| *CST3* | Cystatin C | 110 | 1.05 | 89 | 0.97 |
| *SERPINE1* | Plasminogen activator inhibitor 1 | 120 | 0.66 | 187 | 1.35 |
| *TIMP1* | Metalloproteinase inhibitor 1 | - | - | 56 | 0.26 |
| *PRSS23* | Serine protease 23 | 35 | 0.29 | - | - |
| *CTSB* | Cathepsin B | - | - | 36 | 0.15 |
| *SRGN* | Serglycin | 112 | 5.41 | 109 | 0.85 |
| | Extracellular matrix | | | | |
| *LGALS3BP* | Galectin-3-binding protein | 150 | 0.7 | 268 | 0.65 |
| *FN1* | Fibronectin | 186 | 0.19 | 279 | 0.28 |
| *THBS1* | Thrombospondin-1 | 182 | 0.56 | 271 | 0.65 |
| *EFEMP1* | EGF-containing fibulin-like extracellular matrix protein-1 | 49 | 0.37 | 100 | 0.47 |
| *HSPG2* | Basement membrane-specific heparan sulfate proteoglycan core protein | - | - | 46 | 0.04 |
| | | - | - | 38 | 0.01 |
| *LAMA5* | Laminin 5 | - | - | 47 | 0.11 |
| *EDIL3* | EGF-like repeat and discoidin I-like domain-containing protein 3 | | | | |

**Table S3. Tandem mass spectrometry (MS/MS) endothelial cell secretome analysis**
MS/MS analysis was performed on human brain endothelial cells (hCMEC/D3) conditioned media and compared to the HEK-293T one. Shared hits were removed from the list. Peptide mass tolerance was 20 and 30 parts per million for the peptidome and the proteome respectively, and the fragment mass tolerance was 0.3 Da for both experiments. The sum of the highest ions score for each distinct detected sequence (Score) and the exponentially modified protein abundance index (*em*PAI) are indicated for each of the 22 sequences identified either in the peptidome or proteome analysis.

**A**

| Conditions | Solubility (mM) | Solubility ($mg.ml^{-1}$) |
|---|---|---|
| saline (NaCl 0.9%) | >0.73 | >1.27 |
| saline (15%) + kolliphor HS15 | >0.69 | >1.20 |
| saline (10%) + kolliphor EL | >0.60 | >1.04 |
| saline (10%) + hydroxypropyl-β-cyclodextrin | >0.69 | >1.20 |
| saline (15%) + kolliphor HS15 + PEG (10%) + ethanol (5%) | >0.73 | >1.27 |

**B**

| Location | Plasma | Brain |
|---|---|---|
| **Half-life** (min) | 142+/-22 | n.d. |
| **AUC** | 88391+/-731 ($min.ng.ml^{-1}$) | 21633+/-2742 (min.ng) |
| **Time Cmax** (min) | 10 | 10 |
| **Cmax** ($\mu g.ml^{-1}$) | 1.1 | 0.26 |
| **Cmax** ($\mu M$) | 0.6 | 0.14 |

**Table S4. Pharmacodynamics of MM54 compound after intraperitoneal administration**

**A.** MM54 compound (1 mg) was re-suspended in the different vehicles for 24h, RT, under gentle rotation. Solutions were analysed by HPLC and concentrations calculated using DMSO-re-suspended MM54 as standard. **B.** MM54 compound was solubilized in water and administered intraperitoneally (IP) at the dose of 3.6 mg/kg to healthy mice. Mice (3/group) were sacrificed at 10 min, 30 min, 60 min, 2h, 4h, 6h, 8h and 24h post-injection. Plasma and brains were collected and further processed for LC-MS/MS analysis. B/P index is 0.24. AUC is area under the curve. n.d. stands for 'not able to be determined'.

| Total blood count | DMSO | | MM54 | |
|---|---|---|---|---|
| **Leukocytes** | | | | |
| White blood cells (K/µl) | 10,6 | ± 1,2 | 11,2 | ± 1,3 |
| Neutrophils (K/µl) | 3,07 | ± 0,29 | 2,56 | ± 0,66 |
| Lymphocytes (K/µl) | 5,90 | ± 0,86 | 7,50 | ± 0,51 |
| Monocytes (K/µl) | 0,47 | ± 0,06 | 0,42 | ± 0,11 |
| Eosinophils (K/µl) | 0,86 | ± 0,10 | 0,52 | ± 0,28 |
| Basophils (K/µl) | 0,31 | ± 0,04 | 0,19 | ± 0,12 |
| **Erythrocytes** | | | | |
| Red blood cells (M/µl) | 6,8 | ± 0,9 | 7,5 | ± 0,3 |
| Haemoglobin (g/dl) | 8,9 | ± 1,2 | 9,9 | ± 0,6 |
| Hematocrit (%) | 31,8 | ± 4,3 | 35,4 | ± 1,9 |
| Mean corpuscular volume (fl) | 46,4 | ± 0,2 | 47,2 | ± 0,4 |
| Mean corpuscular haemoglobin (pg) | 13,0 | ± 0,0 | 13,2 | ± 0,2 |
| Mean corpuscular haemoglobin concentration (g/dl) | 27,9 | ± 0,1 | 27,9 | ± 0,3 |
| Red cell distribution width (%) | 15,7 | ± 0,2 | 16,1 | ± 0,1 |
| **Thrombocytes** | | | | |
| Platelet (K/µl) | 314 | ± 28 | 301 | ± 27 |
| Mean platelet volume (fl) | 4,30 | ± 0,07 | 4,45 | ± 0,16 |

**Table S5. Blood analysis after MM54 treatment**
In order to test potential toxic effects of MM54 *in vivo*, C57Bl/6J female mice were administered 2 mg/kg of MM54 or DMSO vehicle, bi-weekly for 4 weeks. At sacrifice, blood was taken for analysis and the heart, kidney, aorta and liver removed, weighed and fixed for histological analysis (as shown in Figure S4). n=4 mice/group, mean±SEM. M: $10^6$, K: $10^3$.