The novel CD19-targeting antibody-drug conjugate huB4-DGN462 shows improved anti-tumor activity compared to SAR3419 in CD19-positive lymphoma and leukemia models

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The novel CD19-targeting antibody-drug conjugate huB4-DGN462 shows improved anti-tumor activity than SAR3419 in CD19-positive lymphoma and leukemia models

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Supplementary materials
Supplementary Table S1. Anti-tumor activity of huB4-DGN462 and SAR3419 in B-cell lymphomas.

| Cell Line | Histology | huB4-DGN462 IC50 (pM) | DGN462 IC50 (pM) | Apoptosis, huB4-DGN462 | SAR3419 IC50 (pM) | Apoptosis, SAR3419 | CD19 (MFI) | BCL2 translocation | MYC translocation | inactive TP53 |
|-----------|-----------|------------------------|------------------|------------------------|------------------|-------------------|-----------|-------------------|-----------------|--------------|
| DB        | GCB-DLBCL | 800                    | 800              | 0                      | 2000             | 0                 | 1174      | yes*              | no ^            | yes ^ 1      |
| DoHH2     | GCB-DLBCL | 0.2                    | 0.1              | 1                      | 3000             | 1                 | 1483      | yes ^ 2           | yes ^            | no ^ 3        |
| ESKOL     | MZL       | 12                     | 0.1              | 1                      | 8000             | 1                 | 366       | no ^              | no ^            | no ^          |
| Farage    | GCB-DLBCL | 0.8                    | 0.8              | 1                      | 20               | 1                 | 3858      | no ^ 4            | no ^            | yes ^ 5       |
| Granta 519| MCL       | 40                     | 0.1              | 1                      | 3000             | 1                 | 392       | no ^ 6            | no ^            | yes ^ 7       |
| HAIR-M    | MZL       | 100                    | 0.1              | 0                      | 5000             | 1                 | 92        | no ^              | no ^            | no ^          |
| HBL-1     | GCB-DLBCL | 750                    | 750              | 1                      | 8000             | 0                 | 346       | no ^              | no ^            | yes ^ 8       |
| HC-1      | MZL       | 30                     | 0.1              | 1                      | 5000             | 1                 | 210       | no ^              | no ^            | no ^ 10       |
| JeKo-1    | MCL       | 100                    | 0.1              | 0                      | 12000            | 0                 | 198       | no ^              | no ^            | yes ^ 9       |
| JVM-2     | MCL       | 50                     | 50               | 0                      | 3000             | 0                 | 65        | no ^              | no ^            | no ^          |
| KarPas-1106| PMBCL   | 200                    | 10               | 1                      | 10000            | 1                 | 2966      | no ^              | no ^            | no ^          |
| KarPas-1718| MZL     | 5                      | 0.1              | 1                      | 1500             | 1                 | 586       | no ^              | no ^            | yes ^ 11      |
| KarPas-422| GCB-DLBCL | 8000                  | 3000             | 1                      | 2000             | 1                 | 1913      | yes 12, 13        | no ^            | yes 10, 12    |
| MAVER-1   | MCL       | 50                     | 0.1              | 1                      | 2000             | 1                 | 1799      | yes ^              | yes ^ 14       | yes ^ 15      |
| MEC-1     | CLL       | 1500                   | 2                | 1                      | 40000            | 0                 | 712       | no ^              | yes ^ 15       | yes ^ 16      |
| Mino      | MCL       | 50                     | 0.1              | 1                      | 5000             | 1                 | 1365      | yes ^              | yes ^ 16       | yes ^ 18      |
| OCI-LY-1  | GCB-DLBCL | 12                     | 200              | 1                      | 600              | 1                 | 1544      | yes 12, 17, 18    | no ^            | yes 18       |
| OCI-LY-10 | ABC-DLBCL | 0.75                   | 0.1              | 1                      | 3500             | 1                 | 627       | no 18, 19         | no ^            | yes 15       |
| OCI-LY-18 | GCB-DLBCL | 3                      | 0.75             | 1                      | 1500             | 1                 | 1286      | yes ^              | yes ^ 18       | yes 18       |
| OCI-LY-19 | GCB-DLBCL | 0.75                   | 0.1              | 1                      | 1200             | 1                 | 4342      | yes ^ 20          | no 18, 21      | yes 15       |
| OCI-LY-3  | ABC-DLBCL | 30                     | 5                | 1                      | 700              | 0                 | 156       | no ^              | no ^ 18, 22    | yes 18       |
| OCI-LY-7  | GCB-DLBCL | 50                     | 50               | 1                      | 5000             | 0                 | 984       | no ^              | yes ^ 12, 18   | yes 18       |
| OCI-LY-8  | GCB-DLBCL | 3                      | 3                | 1                      | 50               | 0                 | 961       | yes 17, 18, 20    | yes ^ 18       | yes 15       |
| PCL12     | CLL       | 3000                   | 1000             | 1                      | 10000            | 1                 | 634       | no ^              | yes 3          | no ^ 23      |
| Pfeiffer  | GCB-DLBCL | 2500                   | 2500             | 0                      | 8000             | 0                 | 997       | yes **             | no ^            | yes 15       |
| RCK8      | ABC-DLBCL | 500                    | 500              | 1                      | 6000             | 0                 | 1791      | no ^              | no ^            | yes 15       |
| Rec-1     | MCL       | 40000                  | 1500             | 0                      | 15000            | 0                 | 312       | no ^              | yes 15         | yes 15       |
| RI-1      | ABC-DLBCL | 3000                   | 2000             | 0                      | 4500             | 1                 | 358       | no ^              | yes 15         | yes 15       |
| SP-49     | MCL       | 5                      | 1.5              | 0                      | 2                | 0                 | 1479      | no ^              | yes 15         | yes 15       |
| Code    | Type          | Viable (%) | Cell Type | IC50 (mg/mL) | IC50 (ng/mL) | IC50 (U/mL) | qRT-PCR | FISH |
|---------|---------------|------------|-----------|--------------|--------------|-------------|---------|------|
| SP-53   | MCL           | 30         | 0.1       | 2000         | 1            | 2397        | no      |      |
| SSK41   | MZL           | 1000       | 800       | 1500         | 1            | 507         | no      |      |
| SU-DHL-10| GCB-DLBCL    | 2000       | 2000      | 100          | 1            | 1109        | yes     | yes  |
| SU-DHL-16| GCB-DLBCL    | 200        | 10        | 7000         | 1            | 15          | no      |      |
| SU-DHL-2 | ABC-DLBCL    | 200        | 200       | 7000         | 1            | 68          | no^     |      |
| SU-DHL-4 | GCB-DLBCL    | 2000       | 2000      | 1            | 7000         | 9568        | yes^    | yes  |
| SU-DHL-5 | GCB-DLBCL    | 3          | 0.1       | 800          | 1            | 2086        | no      |      |
| SU-DHL-6 | GCB-DLBCL    | 1000       | 1000      | 200          | 1            | 746         | yes*    | no^  |
| SU-DHL-8 | GCB-DLBCL    | 40         | 100       | 3000         | 1            | 40          | no*     | yes  |
| TMD8    | ABC-DLBCL    | 3000       | 800       | 1            | 7000         | 402         | no      |      |
| Toledo  | GCB-DLBCL    | 300        | 200       | 1            | 12500        | 2306        | yes^    | yes  |
| U2932   | ABC-DLBCL    | 700        | 700       | 1            | 4000         | 465         | no*     | no^  |
| UPN1    | MCL           | 2          | 1         | 1000         | 1            | 537         | no*     | yes  |
| VAL     | GCB-DLBCL    | 200        | 200       | 1            | 2500         | 2158        | yes*    | yes  |
| VL51    | MZL           | 100        | 0.1       | 1            | 10000        | 20          | no      |      |
| WSU-DLCL2| GCB-DLBCL   | 3500       | 2500      | 1            | 2500         | 1134        | yes*    | no^  |
| Z-138   | MCL           | 5          | 0.1       | 0            | 5000         | 157         | yes     |      |

GCB-DLBCL, germinal center B-cell type diffuse large B-cell lymphoma; ABC-DLBCL, activated B-cell like diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia; PMBCL, primary mediastinal large B-cell lymphoma; n.d., not determined.; *, as described in the catalogue of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (www.dsmz.de); **, as described in the catalogue of the American Type Culture Collection (ATCC)(http://www.lgcstandards-atcc.org); § FISH, XL MYC BA Triple-color Probe, D-6030-100-TC (MetaSystems Probes GmbH, Germany); ^ FISH using the XL t(8;14) Dual Fusion Probe, D-5008-100-OG (MetaSystems Probes GmbH). FISH analyses were performed as previously described. 

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**Supplementary Table S2.** Anti-tumor activity of huB4-DGN462 and SAR3419 in six acute lymphoblastic leukemia cell lines. ABC, antibody binding capacity

| Cell line     | CD19 ABC (x1,000) | huB4-DGN462 | SAR3419 |
|---------------|-------------------|-------------|---------|
| RS4;11        | 32                | 10.2        | 2,117   |
| NALM-6        | 25                | 8.4         | 1,260   |
| TOM-1         | 16                | 15.6        | 3517    |
| Mutz-5        | 13                | 7.2         | 6,259   |
| BALL-1        | 12                | 4.0         | 9,285   |
| GRANTA-452    | 6                 | 9.1         | 5,685   |

ABC, antibody binding capacity
Supplementary Figure S1. huB4-DGN462 and DGN462 induce apoptosis and G2-M arrests in lymphoma cell lines. A. Cell cycle distribution after huB4-DGN462, DGN462 treatment (8pM or 50pM) or DMSO (72 hours). B. Representative histograms of cell cycle distribution and (C) induction of apoptosis in DOHH2 and FARAGE cells treated with huB4-DGN462, DGN462 treatment (8pM or 50pM) or DMSO (72 hours).
Supplementary Figure S2. The in vitro antitumor activity of huB4-DGN462 and SAR3419 are correlated across B-cell lymphoma lines. Y-axis, Log10 IC50 values of SAR3419 (pM). X-axis, Log10 IC50 values of huB4-DGN462 (pM). R, correlation coefficient. P, p-value.
Supplementary Figure S3. Paired line plots showing the higher *in vitro* antitumor activity of huB4-DGN462 than SAR3419. Upper plot shows the differences in median IC50 values by histotype as detailed in Table 1. Lower plot shows the differences for each individual cell line as detailed in Table 1. Median IC50 values by histotype as detailed in Supplementary Table S1. GCB-DLBCL, germinal center B-cell type diffuse large B-cell lymphoma; ABC-DLBCL, activated B-cell like diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia; PMBCL, primary mediastinal large B-cell lymphoma.
Supplementary Figure S4. The *in vitro* antitumor activity of SAR3419 is correlated with surface CD19 expression, but not CD19 RNA levels in B-cell lymphoma lines. Y-axis, CD19 expression levels (Log10) as measured by flow cytometry analysis (upper panel), microarray analysis (middle panel) or next generation sequencing (lower panel). X-axis, Log10 IC50 values of SAR3419 (pM). R, correlation coefficient. P, p-value.
Supplementary Figure S5. The in vitro antitumor activity of huB4-DGN462 is not correlated to CD19 expression in B-cell lymphoma lines. Y-axis, CD19 expression levels (Log10) as measured by flow cytometry analysis (upper panel), microarray analysis with Illumina arrays (middle panel) or NGS with HTG platform (lower panel). X-axis, Log10 IC50 values of huB4-DGN462 (pM). R, correlation coefficient. P, p-value.
Supplementary Figure S6. DLBCL cell of origin, CD19 expression and sensitivity to huB4-DGN462 or SAR3419. A, Sensitivity to SAR3419 but not to huB4-DGN462 is affected by the DLBCL cell of origin. B, GCB-DLBCL cell lines express higher CD19 levels than ABC-DLBCL cell lines. CD19 expression levels were measured (Log10) by flow cytometry analysis (left panel), microarray analysis with Illumina arrays (central panel) or NGS with HTG platform (right panel).
Supplementary Figure S7. Body weight changes after exposure to vehicles, huB4-DGN462 (left panels) or to SAR3419 (right panels) in subcutaneous (upper panel) or disseminated (lower panel) DLBCL xenograft models as shown in Figure 2. Body weights were measured twice weekly.
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