Patient-derived lymphoma xenografts (PDX) in immune-deficient nude mice and primary 3D cell cultures (PD3D®) as pre-clinical toolboxes

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Background

Aggressive B-cell and T-cell lymphomas constitute a substantial proportion of all human malignant Non-Hodgkin lymphomas (NHL) and pose an important clinical challenge. Given the fact that these lymphomas frequently develop resistance to chemotherapy and are associated with high relapse rates, pre-clinical PDX models could provide a valuable tool to understand the molecular mechanisms leading to treatment failure.

We have received four patient tumour tissue samples from aggressive NHLs, two of which were diffuse large B-cell lymphoma (DLBCL) samples from the peripheral blood and two were Burkitt lymphoma samples from fine needle biopsies. We successfully established PDX models and in parallel PD3D models from these tumours.

Methods

The patient-derived tumours were transplanted subcutaneously into immune-deficient NOD scid gamma mice. Within 80 days, palpable tumours were grown and passaged via the same route to immune-deficient NMRI nu/nu mice. Around after five passages, these lymphoma models were used in a chemosensitivity assay. When the tumour volume reached > 0.1 cm^3, treatment with cyclophosphamide, vincristine, rituximab and gemcitabine was started. For the establishment of PD3D, primary cell suspension (one of each type of NHL) was seeded on a Matrigel layer and was grown for at least two weeks. For chemosensitivity testing, lymphoma spheroids from single cell suspension were formed during four days and subsequently treated with vincristine for eight days at different concentrations.

Results

Immunohistochemistry & Chemosensitivity

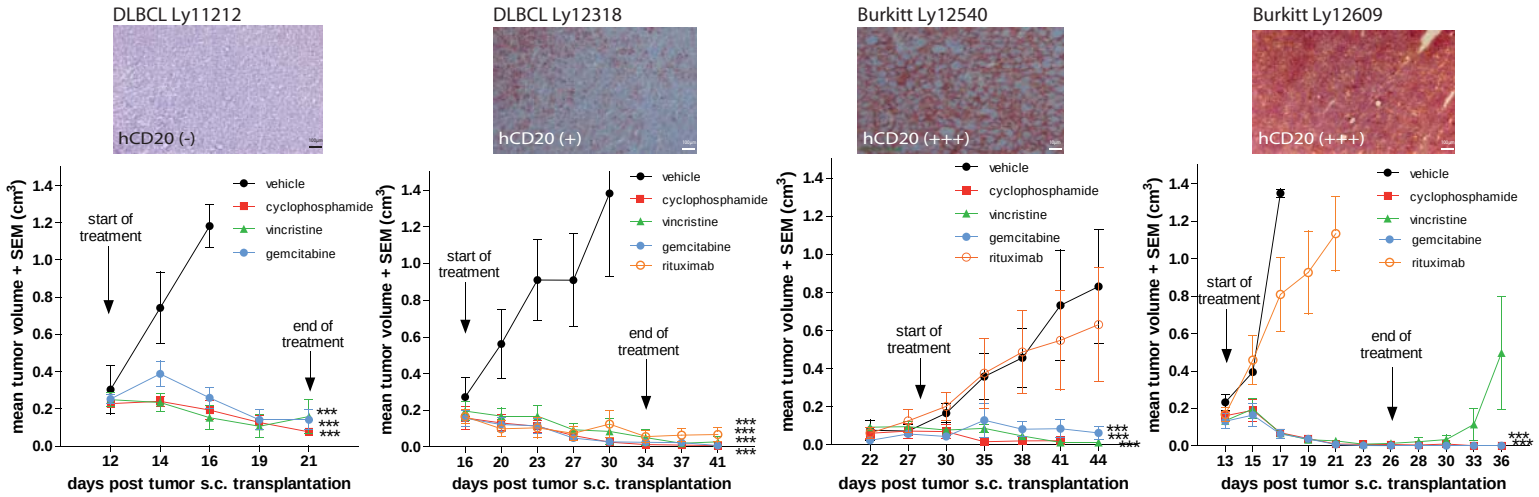
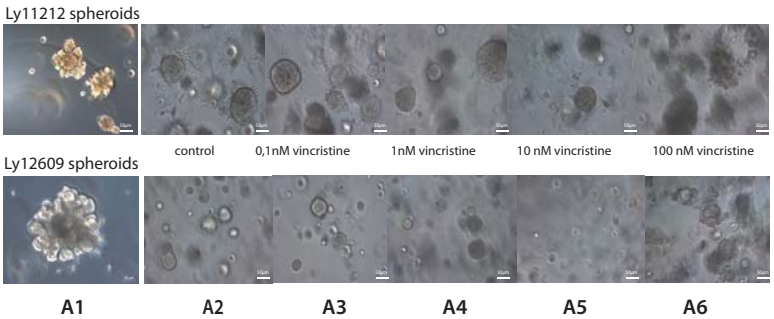


Figure 1

Staining of human CD20 surface molecule expressed on precursors and mature B-cells, but not on plasma cells. DLBCL Ly11212 without any membraneous staining reaction, DLBCL Ly12318 shows a moderate staining and both Burkitt lymphomas Ly12540 and Ly12609 show a strong membraneous staining reaction.

Tumour growth curves (n=5 mice/group) under treatment with chemotherapeutics and rituximab are shown. *** P-value < 0.001 when compared to vehicle group (NaCl, 0.9%) by 2way ANOVA. Ly11212 as hCD20 negative DLBCL was not treated with rituximab, whereas the remaining three lymphoma models (hCD20 positive) were treated with rituximab.

3D cell culture



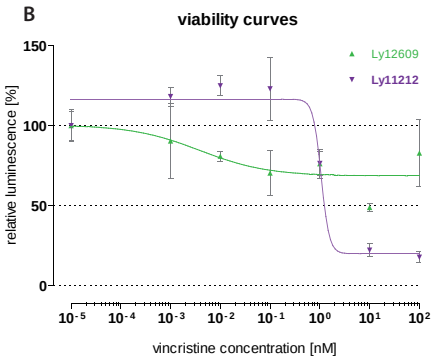


Figure 2

A1: Micrographic representation of spheroids after several weeks of growing.

A2: Spheroids formed four days after seeding.

A3-A6: Morphological alterations of spheroids after eight days of treatment with vincristine at four different concentrations.

B: Viability curves from both NHL-PD3D models according to the treatment described before, confirm the drug effect observed in the light microscopy pictures.

Conclusions

We successfully established four PDX models with solid NHL-tumours on immune-deficient mice as well as the corresponding PD3D models. Both model systems are reproducible.

The best inhibition on tumour growth resulted from treatments with gemcitabine, cyclophosphamide and vincristine as monotherapy in all four NHL-PDX models. Those responses are clinically considered as partial up to complete remission and reflect the clinical situation.

However, in the clinic the NHL were treated by CHOP therapy (cyclophosphamide, doxorubicine, vincristine and prednisone combined) with and without rituximab (R-CHOP). In our chemosensivity assays, only the model DLBCL Ly12318 partially positive for hCD20 showed a response to rituximab whereby both Burkitt lymphomas, strong hCD20 positive, did not show any response to rituximab. These results indicate that a single treatment with rituximab did not reduce the tumour growth and/ or that a resistance to rituximab was developed.

Regarding the PD3D models, the efficacy of vincristine as monotherapy correlates with the results from the chemosensitivity in immune-deficient mice.

These preliminary findings indicate that our NHL-PDX models can be supported by PD3D to investigate cellular and molecular mechanism of drug sensitivity and resistance, thereby providing a valuable pre-clinical tool for developing new therapeutic approaches. In further chemosensitivity assays, the clinical treatment of CHOP and R-CHOP on NHL-PDX in comparison to NHL-PD3D will be tested.

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