Enhanced efficacy of therapy of anti-CD20 antibody with Locked Nucleic Acid antisense oligonucleotide targeting Bcl-2 in human Burkitt's lymphoma xenografts

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RNA Medicines for the 21st Century

Background

Cell survival by abolishing programmed cell death in cancer cells has been closely linked to high Bcl-2 expression. The therapeutic potential of reducing Bcl-2 in cancer cells has been documented and resistance to existing cancer therapies have been linked to Bcl-2.

Materials and Methods

The RNA antagonist, SPC2996, is a 16-mer oligonucleotide incorporating Locked Nucleic Acid (LNA) with unique high-affinity binding to Bcl-2 mRNA and enhanced resistance to nuclease digestion. In cell cultures, SPC2996 shows potent, specific and long-lived reduction in Bcl-2 mRNA and protein levels with an IC50 in the low nanomolar range. In primates substantial down-regulation of Bcl-2 mRNA was observed in tissues after intravenous administration of SPC2996. SPC2996 has completed a phase I/II trial in CLL where a dose response effect of SPC2996 was observed with higher doses giving improved effects on lymphocyte counts, lymph nodes, time to progression and overall responses.

RESULTS

Here we report on the anti-tumour activity of SPC2996 alone and in combination with Rituximab in SCID mice bearing disseminated Raji or Namalwa human Burkitt's lymphoma. The Raji cell line has a high CD20 expression and shows sensitivity to Rituximab while the more aggressive Namalwa cell line has very low CD20 expression and shows resistance to Rituximab. SPC2996 was administered IV daily at 5mg/kg for 14 consecutive days while Rituximab was dosed IV twice weekly at suboptimal doses for 3 weeks. All treatments were started at day 4 after tumour cell injection (xenografting). A scrambled oligonucleotide with no target in the human transcriptome was used as negative control.

• In the Raji model the combination of SPC2996 plus Rituximab showed synergistic effect with significant longer survival than either treatment alone and a T/C% value of 331.0 compared to Rituximab plus the scrambled control oligonucleotide.

• Analysis of the bone marrow at day 18 after Raji tumor cell injection showed a significant reduction in the percentage of human tumor cells from 27.7% in mice treated with the scrambled control oligonucleotide to 1.3% with SPC2996 alone and no signal above background level with the combination of SPC2996 plus Rituximab.

•In the Namalwa model Rituximab alone had no significant effect on survival while SPC2996 alone showed significant prolonged survival with a T/C% value of 143.2 compared to Rituximab plus control oligonucleotide and 166.3 compared to saline. The combination of SPC2996 plus Rituximab significantly prolonged the survival even further with a T/C% value of 195.5 compared to Rituximab plus oligonucleotide.

•The percentage of human tumor cells in the bone marrow at day 14 after Namalwa tumour cell injection showed a reduction from 14.5% in mice treated with the scrambled control oligonucleotide to a level slightly above the background staining with both SPC2996 alone and in combination with Rituximab while Rituximab alone showed 6.6% human tumor cells in the bone marrow.

Conformation of Oxy LNA versus DNA and RNA

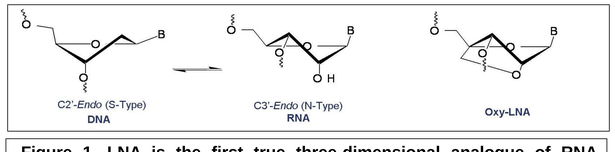

Figure 1. LNA is the first true three-dimensional analogue of RNA (ribonucleic acid). The conformation of the ribose sugar in LNA is 'locked' in the RNA shape by virtue of its rigid bicyclic structure. When incorporated into oligonucleotides, LNA conveys dramatically enhanced binding affinity to complementary RNA sequences. Drug molecules with multiple LNA substitutions therefore have truly outstanding potencies.

Study design - Raji

Group	No animals	Treatment	Route	Dose (mg/kg)	Treatment schedule	Day of treatment	Combined treatment	Route	Dose (mg/kg)	Treatment schedule	Day of treatment
1	12*	Saline	IV	-	Q1Dx14	D4-D17	-	-	-	-	-
2	12*	SPC2996	IV	5	Q1Dx14	D4-D17	-	-	-	-	-
3	12*	Control oligo	IV	10	Q1Dx14	D4-D17	-	-	-	-	-
4	12*	-	-	-	-	-	Rtx	IV	1	TWx3	D4, D7, D11, D14, D18, D21
5	12*	SPC2996	IV	5	Q1Dx14	D4-D17	Rtx	IV	1	TWx3	D4, D7, D11, D14, D18, D21
6	12*	Control oligo	IV	10	Q1Dx14	D4-D17	Rtx	IV	1	TWx3	D4, D7, D11, D14, D18, D21
7	12*	-	-	-	-	-	Rtx	IV	10	TWx3	D4, D7, D11, D14, D18, D21

*** 4 animals from each group sacrificed at D18 for tumour cell engraftment analysis**

Figure 2. The treatment schedule of the disseminated Raji Burkitt's lymphoma model on the anti-tumour activity of SPC2996 in combination with Rituximab (Rtx). Rituximab was dosed in a suboptimal dose of 1mg/kg twice weekly for the combination with SPC2996. A scrambled control oligo was used as a negative control. Four animals in each group were sacrificed at day 18 for analysis of tumour cell engraftment into the bone marrow.

Survival data - Raji

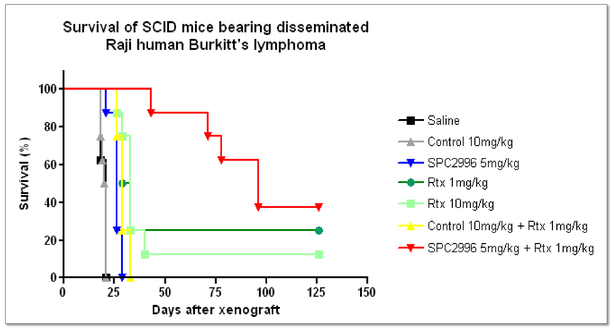

Figure 3. Survival curves of the different groups in the Raji study. The saline and control oligo groups died first. SPC2996 administered alone showed modest but significant improvement on survival compared to the saline group but not compared to the Rituximab treatments. In contrast the combination of SPC2996 plus Rituximab showed a marked and highly significant improvement of antitumour activity compared to Rituximab alone and plus control oligo. At day 60 only one animal in the SPC2996 plus Rituximab group had died why the study was continued until day 126. No tumour cells were found in the bone marrow of surviving animals at day 126.

Bone Marrow engraftment data - Raji

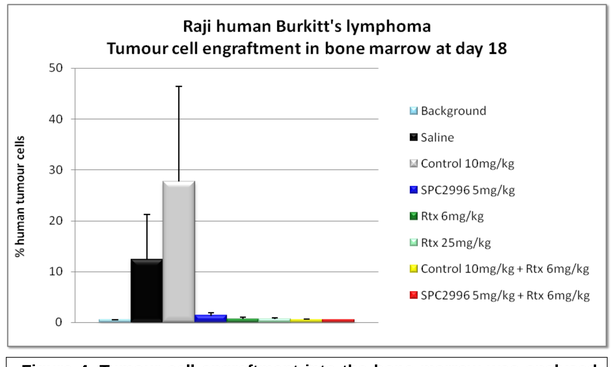

Figure 4. Tumour cell engraftment into the bone marrow was analysed at day 18 after Raji xenografting. No tumour cells were detected in the bone marrow from mice treated with Rituximab alone or in combination with SPC2996 and control oligo. In contrast, a similar level were detected in bone marrow of mice treated with control oligo alone and saline. A slight level of tumour cells were detected in bone marrow of mice treated with SPC2996 alone.

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Statistics Raji, day 126

Group	Treatment	Median Survival
1	Saline	21
2	SPC2996 5mg/kg	26
3	Control oligo 10mg/kg	21
4	Rtx 1mg/kg	31
5	Rtx 1mg/kg + SPC2996 5mg/kg	96
6	Rtx 1mg/kg + control oligo 10mg/kg	29
7	Rtx 10mg/kg	33

T/C%	Rtx 1mg/kg + control oligo 10mg/kg	SPC2996 5mg/kg	Rtx 1mg/kg + SPC2996 5mg/kg
Saline	138.1	123.8	457.1
Rtx 1mg/kg + control oligo 10mg/kg	-	83.9	331.0

P value (Log rank test)	Rtx 1mg/kg + control oligo 10mg/kg	SPC2996 5mg/kg	Rtx 1mg/kg + SPC2996 5mg/kg
Saline	0.0001	0.0006	0.0001
Rtx 1mg/kg + control oligo 10mg/kg	-	0.0290**	0.0009

**** Rtx + control oligo treatment prolongs the survival compared to SPC2996**

Figure 5. The statistics of the Raji study showing the median survival and T/C% values of the different treatments, and the p-values of the survival curves. The median survival of the saline and control oligo groups were 21 days while SPC2996 alone prolonged survival until 26 days and the combination of SPC2996 plus Rituximab prolonged survival to 96 days giving a T/C% of 331.0 compared to Rituximab plus control oligo. The T/C% of SPC2996 alone compared to saline was just below 125% and less effective than Rituximab plus control oligo.

Study design - Namalwa

Group	No animals	Treatment	Route	Dose (mg/kg)	Treatment schedule	Day of treatment	Combined treatment	Route	Dose (mg/kg)	Treatment schedule	Day of treatment
1	12*	Saline	IV	-	Q1Dx14	D4-D17	-	-	-	-	-
2	12*	SPC2996	IV	5	Q1Dx14	D4-D17	-	-	-	-	-
3	12*	Control oligo	IV	10	Q1Dx14	D4-D17	-	-	-	-	-
4	12*	-	-	-	-	-	Rtx	IV	6	TWx3	D4, D7, D11, D14, D18, D21
5	12*	SPC2996	IV	5	Q1Dx14	D4-D17	Rtx	IV	6	TWx3	D4, D7, D11, D14, D18, D21
6	12*	Control oligo	IV	10	Q1Dx14	D4-D17	Rtx	IV	6	TWx3	D4, D7, D11, D14, D18, D21
7	12*	-	-	-	-	-	Rtx	IV	25	TWx3	D4, D7, D11, D14, D18, D21

*** 4 animals from each group sacrificed at D14 for tumour cell engraftment analysis**

Figure 6. The treatment schedule of the disseminated Namalwa Burkitt's lymphoma model on the anti-tumour activity of SPC2996 in combination with Rituximab. Rituximab was dosed in a suboptimal dose of 6mg/kg twice weekly for the combination with SPC2996. A scrambled control oligo was used as a negative control. Four animals in each group were sacrificed at day 14 for analysis of tumour cell engraftment into the bone marrow.

Survival data – Namalwa

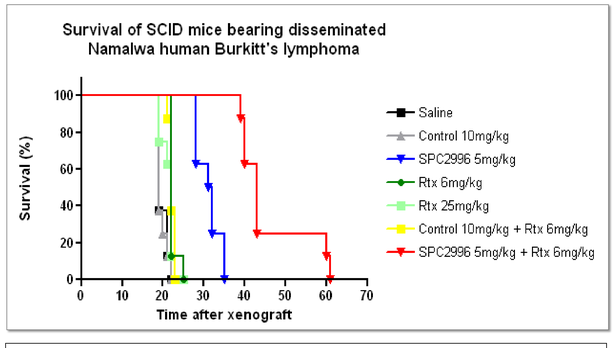

Figure 7. Survival curves of the different groups in the Namalwa Study. The saline and control oligo groups died first followed closely by the Rituximab groups. SPC2996 administered alone displayed a highly significant antitumor activity superior to the antitumor effect of Rituximab with or without control oligo. The combination of SPC2996 plus Rituximab showed a marked and highly significant improvement of the antitumour activity compared to Rituximab alone and SPC2996 alone.

Bone Marrow engraftment data – Namalwa

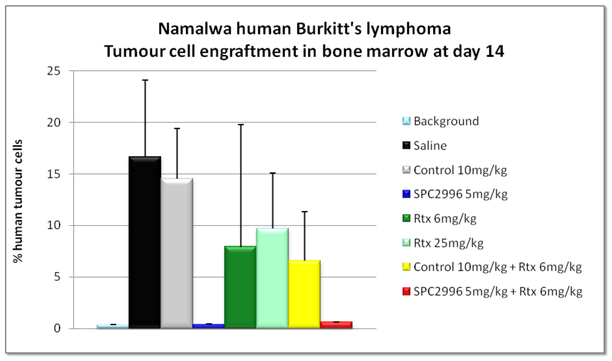

Figure 8. Tumour cell engraftment into the bone marrow was analysed at day 14 after Namalwa xenografting. A slight level of tumour cells were detected in the bone marrow of mice treated with SPC2996 alone and in combination with Rituximab. An increase in the level of tumour cells were observed for mice treated with Rituximab at either concentration. A similar level of tumour cells were detected in the bone marrow of mice treated with control oligo and saline.

Statistics Namalwa, day 61

Group	Treatment	Median Survival
1	Saline	19
2	SPC2996 5mg/kg	31.5
3	Control oligo 10mg/kg	19
4	Rtx 6mg/kg	22
5	Rtx 6mg/kg + SPC2996 5mg/kg	43
6	Rtx 6mg/kg + control oligo 10mg/kg	22
7	Rtx 25mg/kg	22

T/C%	Rtx 6mg/kg + control oligo 10mg/kg	SPC2996 5mg/kg	Rtx 6mg/kg + SPC2996 5mg/kg
Saline	115.8	165.8	226.3
Rtx 6mg/kg + control oligo 10mg/kg	-	143.2	195.5

P value (Log rank test)	Rtx 6mg/kg + control oligo 10mg/kg	SPC2996 5mg/kg	Rtx 6mg/kg + SPC2996 5mg/kg
Saline	0.0402	< 0.0001	< 0.0001
Rtx 6mg/kg + control oligo 10mg/kg	-	< 0.0001	< 0.0001

Figure 9 The statistics of the Namalwa study showing the median survival and T/C% values of the different treatments, and the p-values of the survival curves. The median survival of the saline and control oligo groups were 19 days while SPC2996 alone prolonged survival until 31.5 days giving a T/C% of 166.3 compared to saline and 143.2 compared to Rituximab plus control oligo. The combination of SPC2996 plus Rituximab prolonged survival to 43 days giving a T/C% of 226.3 compared to saline and 195.5 compared to Rituximab plus control oligo.

CONCLUSION

We have here presented data on the LNA containing RNA antagonist SPC2996 targeting Bcl-2 in two different human Burkitt's lymphoma xenograft models.

In the disseminated Raji Burkitt's lymphoma model SPC2996 administered alone displayed a moderate antitumor activity. In contrast SPC2996 showed a marked and highly significant improvement of the antitumour activity when combined with Rituximab compared to Rituximab alone.

In the more aggressive and Rituximab resistant disseminated Namalwa Burkitt's lymphoma model SPC2996 administered alone displayed a highly significant antitumor activity superior to the antitumor effect of Rituximab alone. The combination of SPC2996 plus Rituximab showed a marked and highly significant improvement of the antitumour activity compared to SPC2996 alone and Rituximab alone.