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Preclinical Characterization of YBL-006, a Fully Human Anti-PD-1 Antibody Being Ready for Clinical Studies

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Abstract

Cancer immunotherapy with immune checkpoint inhibitors which enhances T-cell regulatory pathways has provided unprecedented benefit to cancer patients. Programmed death 1 ligand (PD-L1) being expressed by cancer cells binds to programmed death-1 (PD-1) to avoid anti-tumor activity of immune cells. Antibodies to disrupt the interaction have been developed for cancer therapeutics. YBL-006 is a new anti-PD-1 human IgG4 monoclonal antibody. Here, we described *in vitro* & *in vivo* studies to evaluate pharmacological effect of YBL-006 along with non-clinical studies of pharmacokinetics, toxicokinetics & tissue cross reactivity (TCR). YBL-006 was bound specifically to human PD-1 among receptors of B7 family by ELISA. When affinity to recombinant PD-1 of various species was measured using surface plasmon response system, K_D of YBL-006 was 0.372 nM to human PD-1 & 0.070 nM to cynomolgus monkey PD-1 which is higher affinity than nivolumab (1.37 nM & 2.50 nM) & pembrolizumab (1.44 nM & 0.817 nM). It did bind to mouse PD-1 with less affinity than to human PD1, while nivolumab & pembrolizumab did not bind to mouse PD-1. YBL-006 was able to inhibit interaction between PD-1 & both PD-L1 & PD-L2 which was confirmed in competitive binding assay (data not shown). Inhibition of PD-1 by YBL-006 increased the level of IFN-γ in a mixed lymphocyte reaction model. In a syngeneic mouse model of MC38 colon cancer, weight of tumors in mice treated with YBL-006 was less than that in mice with human IgG control by 25.4%. In a humanized mouse model (miXeno mouse) of HCC827 non-small cell lung cancer, volume of tumors in YBL-006-treated mice was smaller (35 % less comparing to control mice at donor A, 36 % less comparing to control mice at donor B) than that in nivolumab-treated mice (24 % less comparing to control mice at donor A and B). Pharmacokinetic analysis in monkey showed that exposures to YBL-006 increased dose-dependently & in a dose-proportional manner. Maximum mean serum concentrations (C_{max}) of YBL-006 were reached (T_{max}) between 0.5- & 1.0-hour post onset of infusion & YBL-006 mean serum concentrations slowly declined at a mean estimated t1/2 value of 70.8–110 hours. Clearance & mean volume of distribution suggest that YBL-006 was mainly distributed throughout the blood. In 1-month IV infusion toxicity study in monkey, there was no toxicity when they were infused up to 100 mg/kg/dose of YBL-006, but anti-drug antibody was observed (7 out of 22 monkeys). There was no potential toxicity in TCR study. YBL-006 is an anti-PD-1 antibody with high affinity, promising anti-tumor activity in animal models, & favorable safety profile. First-inhuman phase I trial to investigate the safety and efficacy of YBL-006 in advanced solid cancer will be held in 2020.

Introduction

- Immune checkpoints in the immune cells are molecules in maintenance of immunologic homeostasis and help to maintain peripheral tolerance to self-molecules.
- There are many immune checkpoint molecules to augment or inhibit an immune response.
- Tumor cells can escape from the attack of immune system through many mechanisms including the expression of immune suppressive molecules on their cell surface (for example, PD-L1 in **Figure 1**), secretion of soluble suppressive factors, and the recruitment of other suppressive immune cells to the tumor microenvironment.
- The monoclonal antibodies to disrupt co-inhibitory immune checkpoint molecules (for example, CTLA-4 or PD-1) have shown to increase a baseline T-cell specific immune response that changes immune cells to attack the tumor (**Figure 1**).
- YBL-006 is a new human IgG4 monoclonal antibody against PD-1 and has a higher affinity to PD-1 receptor than nivolumab or pembrolizumab.
- Non-clinical studies of YBL-006 demonstrated that it has good safety profile and pharmacological activity.
- YBL-006 has potential to be a good oncology therapeutic agent to treat patients who have solid tumors.
- Phase 1 first-in-human (FIH) study for YBL-006 is ongoing in many countries.

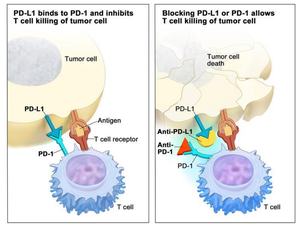


Figure 1. Immune checkpoint inhibitors Checkpoint molecules, such as PD-L1 on tumor cells and PD-1 on T cells, help keep immune responses in check. The binding of PD-L1 to PD-1 keeps T cells from killing tumor cells in the body (left). Blocking the binding of PD-L1 to PD-1 with and immune checkpoint inhibitors (anti-PD-1 or anti-PD-L1) allows the T cells to kill tumor cells (right).
https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immune-checkpoint-inhibitor

Binding Affinity of YBL-006

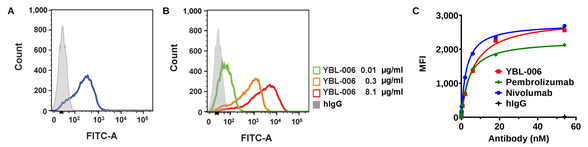


Figure 2. Binding characteristic of YBL-006 to PD-1+/HEK293E cells (A) Flow cytometry analysis of PD-1 which was expressed on PD-1+/HEK293E cells. (B) Flow cytometry analysis of YBL-006 (anti-PD-1 antibody) to PD-1 on the surface of PD-1+/HEK293E kidney cancer cells. (C) Binding profile of YBL-006 and other anti-PD-1 antibodies (nivolumab and pembrolizumab) to PD-1 on the surface of PD-1+/HEK293E cells.

Table 1. Results of kinetic analysis of various species of PD-1 antigen and anti-PD-1 antibodies

PD-1		Human	Cynomolgus monkey	Mouse	Rat
Nivolumab	K_D (nM) *	1.97 ± 0.43	2.35 ± 0.03	No binding	No binding
Pembrolizumab	K_D (nM) *	1.57 ± 0.52	0.72 ± 0.01	No binding	No binding
YBL-006	K_D (nM) *	0.295 ± 0.05	0.068 ± 0.026	5,080 ± 600	No binding

(The results in this table were updated to the latest values.) *Mean ± SD

Functional Assay

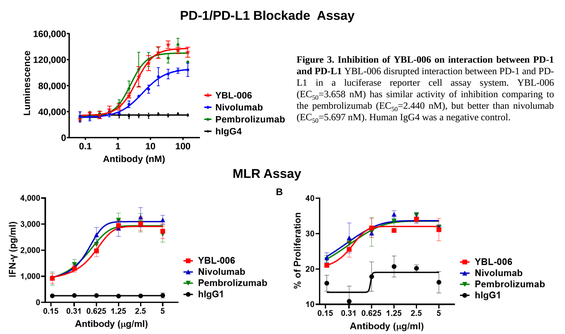


Figure 3. Inhibition of YBL-006 on interaction between PD-1 and PD-L1 YBL-006 disrupted interaction between PD-1 and PD-L1 in a luciferase reporter cell assay system. YBL-006 (EC_{50}=3.658 nM) has similar activity of inhibition comparing to the pembrolizumab (EC_{50}=2.440 nM), but better than nivolumab (EC_{50}=5.697 nM). Human IgG4 was a negative control.

Figure 4. Mixed lymphocyte reaction (MLR) of YBL-006 Human peripheral blood CD8+ T cells were co-cultured for 5 days with allogeneic dendritic cells (DCs) in the presence of the indicated concentration of YBL-006, nivolumab, pembrolizumab, human IgG1. (A) Supernatants were collected at day 5 and IFN-γ produced by CD8+ T cells was measured by ELISA. (B) Representative graph shows the percentages of CTV (CellTrace Violet dye) low stained CD8+ T cells being co-cultured with allogeneic DCs for 5 days.

In vitro Assay for Safety Prediction

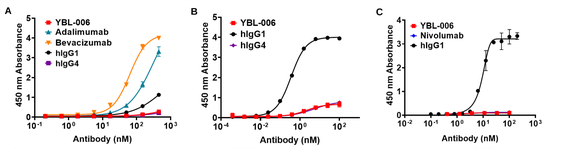


Figure 5. Antibody-dependent cell mediated cytotoxicity (ADCC), antibody-dependent cell mediated phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) of YBL-006. (A) For ADCC assay, binding of YBL-006 to CD16a (FcγRIII) was measured by ELISA. YBL-006 and human IgG4 did not interact with CD16a while human IgG1 and other human IgG1 type antibodies (adalimumab, bevacizumab) had strong interactions with CD16a. (B) For ADCP assay, binding of YBL-006 to CD64 (FcγRI) was measured by ELISA. YBL-006 and human IgG4 did not interact with CD64 at low concentration and moderate interaction at high concentration, but interaction between CD64 and human IgG1 was strong. (C) For CDC assay, binding of YBL-006 to C1q was measured by ELISA. YBL-006 and nivolumab, human IgG4 type antibodies, did not interact with C1q while human IgG1 interact strongly with C1q.

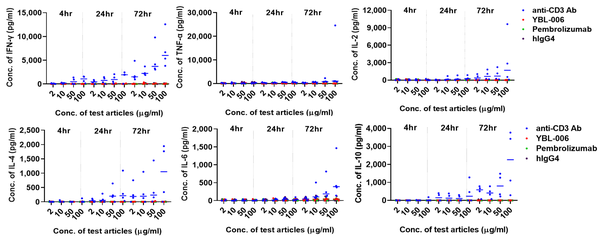


Figure 6. Cytokine release assay of YBL-006 To estimate safety of YBL-006 in a human study, human PBMCs were stimulated with YBL-006 and concentration of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were measured by ELISA. PBMCs were stimulated for 4, 24 and 72 hours and the cytokines in the harvest medium were analyzed. Anti-CD3 antibody was treated as a positive controls and human IgG4 was treated as a negative control. And pembrolizumab was analyzed for comparison with YBL-006. Only anti-CD3 antibody induced the release of the cytokines from PBMC, but PBMC treated with YBL-006 and pembrolizumab did not release the cytokines.

Antitumor Effect of YBL-006 in Murine MC-38 Colon Cancer Model

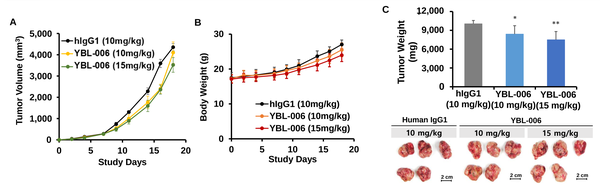


Figure 7-1. Anti-tumor activity of YBL-006 in murine MC-38 colon cancer model Female C57BL/6 mice bearing MC-38 tumors (n=5 mice/group) were treated on days 0, 3, 7, 10, 14, and 17 with YBL-006 or human IgG1. (A) Changes in tumor volume (mm^3). The average tumor volumes treated with YBL-006 (10 mg/kg and 15 mg/kg) were reduced by 5.9% and 19.1% (p<0.01) respectively, comparing to the group treated with human IgG1 (10 mg/kg). (B) Body weight (g). No significant changes in body weight were observed among the mice in all groups during the experiment. (C) Tumor weight (mg) and tumor photos on the last day of experiment (Day 18). Tumor weight of the mice treated with YBL-006 (10 mg/kg and 15 mg/kg) were less than those with human IgG1 (10 mg/kg) (16.4% [p<0.05] and 25.4% [p<0.01] respectively).

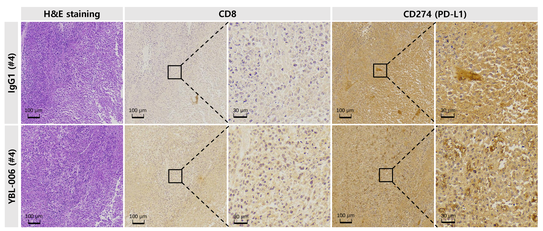


Figure 7-2. Immunohistochemical analysis of CD8 and CD274 (PD-L1) in samples obtained from paraffin-embedded tumor sample Tumors at the end of study (Day 18, **(C) of Figure 7-1**) were harvested and processed for IHC. Sections stained with anti-CD8 or anti-PD-L1 mAbs showed that tumor sample of YBL-006 has more positive signals in both CD8 and PD-L1 than that of IgG1.

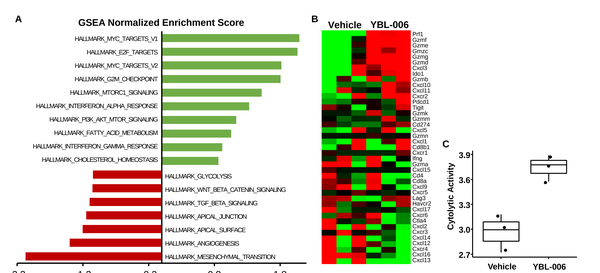


Figure 7-3. Gene-set enrichment analysis from RNA sequencing of tumor tissues (A) Tumors of YBL-006 treatment group enriched with factors of cancer-promoting pathways. (B) Heatmap showed increased expressions of genes associated with local cytolytic activity (e.g. granzyme, perforin) in YBL-006 treatment group. (C) Cytolytic score, measured by geometric mean of expressions of corresponding genes, was significantly higher in YBL-006 treatment group (P < 0.05, box plot).

In Vivo Efficacy of YBL-006 in HCC827 Human NSCLC MiXeno Model

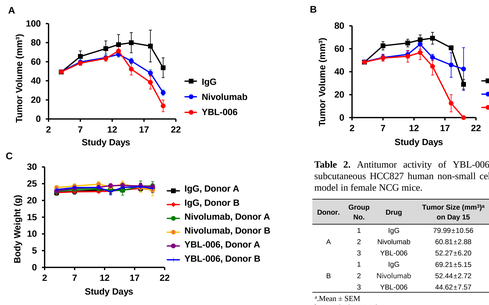


Figure 8. *In vivo* efficacy of YBL-006 in HCC827 human non-small cell lung cancer model in female NCG mice For donor A, the YBL-006 at 5 mg/kg demonstrates significant anti-tumor efficacy (tumor growth inhibition TGI was 35%, p=0.050) compare to vehicle control group. For donor B, the YBL-006 at 5 mg/kg demonstrated anti-tumor efficacy but not significant (TGI was 36%, p=0.063). (A) Mean tumor volume ± SEM (Donor A; YBL-006, nivolumab) (B) Mean tumor volume ± SEM (Donor B; YBL-006, nivolumab). (C) Mean body weight ± SEM.
TGI: Tumor Growth Inhibition; TGI% = (1-Ti/Vi) x 100;Ti as the mean tumor volume of the treatment group on the measurement day; Vi as the mean tumor volume of control group at the measurement day. The T/C value (%) is an indicator of tumor response to treatment, and one of commonly used anti-tumor activity endpoint; T and C are the mean tumor volumes of the treatment and control groups, respectively, on a given day.

Table 2. Antitumor activity of YBL-006 in the treatment of subcutaneous HCC827 human non-small cell lung cancer MiXeno model in female NCG mice.

Donor.	Group No.	Drug	Tumor Size (mm^3)[a] on Day 15	TGI (%)	T/C (%)	P value[b]
A	1	IgG	79.99±10.56	-	-	-
	2	Nivolumab	60.81±2.88	24	76	0.068
	3	YBL-006	52.27±6.20	35	65	0.050
B	1	IgG	69.21±5.15	-	-	-
	2	Nivolumab	52.44±2.72	24	76	0.018
	3	YBL-006	44.62±7.57	36	64	0.063

[a] Mean ± SEM
[b] vs. vehicle control.

In Vivo Efficacy of YBL-006 in the Murine MC-38 Colon Cancer Model in Mice with Human PD-1

Figure 9. Anti-tumor effect of YBL-006 in murine MC-38 colon cancer model in humanized PD-1 mice (A) Change in tumor volume (mm^3). The average tumor volume was significantly reduced in YBL-006 and two other anti-PD-1 groups compared to the IgG group. (B) Change in body weight (g). No significant changes in body weight were observed in all treatment groups during the experiment. (C) Tumor weight (g) on the last day of experiment (Day 28). YBL-006 and two other anti-PD-1 groups significantly reduce tumor weight compared to control IgG group. Mann-Whitney U-test; *p < 0.05, ***p <0.001.

Table 3. Antitumor activity of YBL-006 in the MC-38 colon cancer in humanized PD-1 mice.

28 days	Mean Tumor Weight (g)	T/C (%)	Inhibition (%)	Survival
IgG	4.40	100.00	0	6/7
Pembrolizumab	1.86	42.23	57.77	5/7
Nivolumab	1.47	33.45	66.55	5/7
YBL-006	1.43	32.55	67.45	5/7

Pharmacokinetics

A single intravenous administration of YBL-006 at doses up to 30 mg/kg in male cynomolgus monkeys was well-tolerated and did not result to signs of overt toxicity. Pharmacokinetic analysis showed that exposures to YBL-006 increased dose-dependently and in a dose-proportional manner. Maximum mean serum concentrations (C_{max}) of YBL-006 were reached (T_{max}) between 0.5- and 0.833-hour post onset of infusion. After T_{max} was attained, YBL-006 mean serum concentrations slowly declined at a mean estimated $t_{1/2}$ value ranging from 70.8 to 110 hours. YBL-006 was cleared at a mean rate of 0.505 to 0.547 mL/hour/kg and the mean volume of distribution ranged from 39.8 to 78.3 mL/kg suggesting that YBL-006 was mainly distributed throughout the blood.

Table 4. Mean pharmacokinetic parameters of YBL-006 in male cynomolgus monkey serum on Day 1

Group	Dose Level	C_{max} (μg/mL)		T_{max} (hr)		$T_{1/2}$ (hr)		$AUC_{0\text{-}Tlast}$ (hr*μg/mL)		AUC_{INF_obs} (hr*μg/mL)		Vz_obs (mL/kg)		Cl-obs (mL/hr/kg)	
	(mg/kg)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	3	66.5	5.540	0.500	0.00	110	25.9	4,750	805	6,110	715	78.3	17.1	0.505	0.0590
2	10	237.0	7.330	0.833	0.167	90.5	23.8	19,000	1,710	22,300	1,000	58.0	13.4	0.451	0.0206
3	30	566.0	41.200	0.667	0.167	70.8	49.6	59,400	13,000	61,900	15,300	39.8	20.7	0.547	0.1300

Figure 9. Mean serum concentration of YBL-006 treatment groups in male cynomolgus monkeys on Day 1 Group 1 (YBL-006, 3 mg/kg); Group 2 (10 mg/kg); Group 3 (30 mg/kg)

Table 5. Dose proportionality of YBL-006 in male cynomolgus monkey serum on Day 1

Group	Dose Level	Fold Increase [a]			
	mg/kg	Dose	C_{max}	$AUC_{0\text{-}Tlast}$	AUC_{INF_obs}
1	3	-	-	-	-
2	10	3.3	3.6	4.0	3.6
3	30	3.0	2.4	3.1	2.8
	Overall [b]	10.0	8.5	12.5	10.1

[a] : Fold increase between adjacent doses
[b] : Overall fold increase between high and low dose level

One-month Repeated Toxicology Study and Toxicokinetics

A once weekly intravenous administration of YBL-006 at doses of 10 (Group 2), 30 (Group 3), and 100 mg/kg/dose (Group 4) on 5 occasions (Days 1, 8, 15, 22 and 29) in male and female cynomolgus monkeys was well-tolerated and did not result to signs of overt toxicity. The no observed adverse effect level (NOAEL) was therefore considered to be 100 mg/kg/dose. At the NOAEL, exposures to YBL-006 on Day 22 (represented by the mean C_{max} and $AUC_{0\text{-}Tlast}$) were 2,390 μg/mL and 101,000 hr*μg/mL, respectively in males and 3,520 μg/mL and 220,000 hr*μg/mL, respectively in females. Pharmacokinetic analysis showed that exposures to YBL-006 increased dose-dependently and in a dose-proportional manner. TK analysis suggested that YBL-006 was mainly distributed throughout the blood.

On Day 22 and Day 29, in the 10 mg/kg/dose group (Group 2) sample 2001A male and 2503B female were confirmed positive for anti-YBL-006 antibody. In the 30 mg/kg/dose group (Group 3) samples 3001A and 3002B (males) and 3501A (female) were positive and in the 100 mg/kg/dose group (Group 4) animals 4004C (male) and 4503B (female) were positive. The result suggested that ADA does not depend on dose.

Table 6. Dose proportionality of mean YBL-006 C_{max}, $AUC_{0\text{-}Tlast}$ and AUC_{INF_obs} in cynomolgus monkeys serum for Groups 2 to 4 on Day1 and Day 22

Group	Dose Level	Fold Increase [a]	C_{max} (μg/mL)		Fold Increase [a] C_{max}		$AUC_{0\text{-}Tlast}$ (hr*μg/mL)		Fold Increase [a] $AUC_{0\text{-}Tlast}$		AUC_{INF_obs} (hr*μg/mL)		Fold Increase [a] AUC_{INF_obs}	
	(mg/kg/dose)	(dose)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day 1														
2	10	-	283	183	-	-	16,400	12,800	-	-	27,900	23,100	-	-
3	30	3	856	740	3.0	4.0	46,000	41,100	2.8	3.2	81,600	68,000	2.9	2.9
4	100	3.3	2840	2870	3.3	3.9	153,000	161,000	3.3	3.9	310,000	335,000	3.8	4.9
	Overall [b]	10.0			10.0	15.7			9.3	12.6			11.1	14.5
Day 22 [c]														
2	10	-	307	351	-	-	10,000	20,500	-	-	10,200	26,000	-	-
3	30	3	771	906	2.5	2.6	43,400	38,500	4.3	1.9	53,200	45,300	5.2	1.7
4	100	3.3	2,390	3,520	3.1	3.9	101,000	220,000	2.3	5.7	128,000	450,000	2.4	9.9
	Overall [b]	10.0			7.8	10.0			10.1	10.7			12.5	17.3

[a] : Fold increase between adjacent doses
[b] : Overall fold increase between high and low dose level
[c] : Animals 2001A, 2503B, 3001A, 3002B, 3501A, 4004C and 4503B excluded from mean calculations

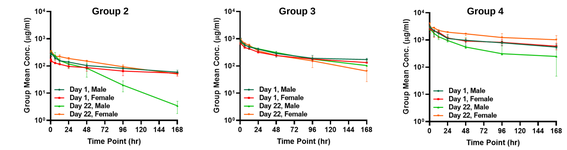
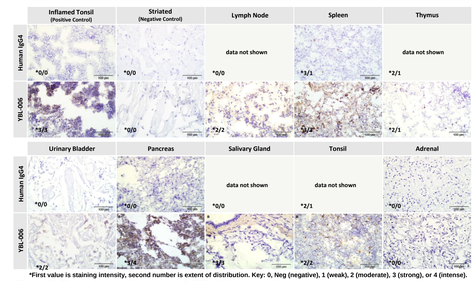


Figure 11. Mean serum concentration of YBL-006 treatment groups in male and female cynomolgus monkeys on Day 1 and Day 22. The $AUC_{0\text{-}Tlast}$ accumulation ratios (Day 22/Day 1) ranged from 0.6 to 1.6 indicated that YBL-006 did not accumulate when administered once weekly intravenous infusion over a period of 30 minutes/occasion to the Cynomolgus monkey over a 4-week period (on Days 1, 8, 15, and 22).

Tissue Cross Reactivity



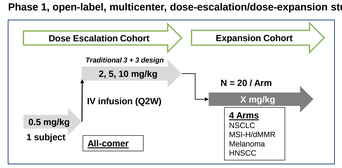
*First value is staining intensity, second number is extent of distribution. Key: 0, Neg (negative), 1 (weak), 2 (moderate), 3 (strong), or 4 (intense).

Figure 12. Tissue cross-reactivity study of YBL-006 in human tissues

Positive tissue cross-reactivity was observed in lymphoid cells of tonsil, thymus, spleen, and lymph nodes from human donors, as well as epithelial glandular cells of urinary bladder, salivary gland, and pancreas from the donors. The staining pattern appeared as multifocal including positive granular membrane staining of lymphocytes (in spleen, thymus, tonsil, and lymph node) as well as cytoplasmic membrane staining of epithelial cell types (in salivary gland, ureter and urinary bladder). Although specific positive staining results suggest that corresponding human tissues may be targeted by YBL-006, this is not necessarily indicative of potential toxicity in man.

Phase I Design

Phase 1, open-label, multicenter, dose-escalation/dose-expansion study of YBL-006.



Endpoints: Safety (DLT)
Recommended dose for phase 2
Efficacy
PK, PD, and ADA

Countries: Republic of Korea,
Australia, Thailand, and Malaysia

Conclusion

- YBL-006 is a new human IgG4 monoclonal antibody against PD-1 and showed better pharmacological characteristics than nivolumab or pembrolizumab.
- YBL-006 anti-cancer activity was observed in many cancer models of mice and gene analysis by RNAseq showed that Immune cytolytic activity was significantly increased by YBL-006 treatment.
- Non-clinical studies of YBL-006 demonstrated that it has good safety profiles.
- Pharmacokinetic and toxicokinetic study demonstrated that YBL-006 has kinetic profiles similar to other antibody drugs.
- YBL-006 has potential to be a good oncology therapeutic agent to treat patients who have solid tumors.
- Phase 1 first-in-human (FIH) study for YBL-006 is ongoing in many countries.

References

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