DT095895, a selective EP_4 receptor antagonist with monotherapy efficacy in syngeneic mouse model(s) and best-in-class properties

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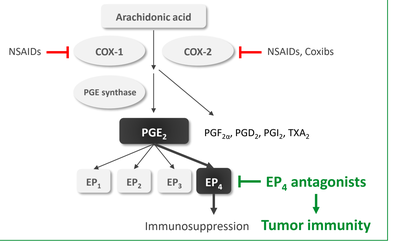
INTRODUCTION

Elevated levels of Prostaglandin E_2 (PGE_2), an eicosanoid synthesized from arachidonic acid by the inducible cyclooxygenase-2 (COX-2), exert **strong immunosuppressive effects** in the **tumor microenvironment**. COX-2-positive solid tumors can use this pathway as a resistance mechanism, especially to escape from the host immune system. Counteracting this immunosuppressive pathway is thought **to restore tumor immunity** and have the potential **to synergize with the anti-tumor effects of immune checkpoint inhibitors** (ICI). Such a combination strategy is highly promising to improve the response rate of patients to immunotherapies and achieve a more efficient, long-lasting, tumor control.

As the use of nonsteroidal anti-inflammatory drugs (NSAIDs) or COX-2 inhibitors (Coxibs) in cancer therapy proved to be non applicable due to safety concerns, there is a need to develop safer alternatives, especially by inhibiting downstream targets. **PGE_2 immunosuppressive effects are largely mediated by the EP_4 receptor, expressed on multiple immune cells. The development of antagonists of the EP_4 receptor is thus a promising strategy to inhibit the PGE_2-induced immunosuppression in the tumor microenvironment and to restore tumor immunity**.

A novel series of EP_4 receptor antagonists, with improved pharmacokinetic properties when compared to the EP_4 receptor antagonists currently being evaluated in clinical trials, has been developed. A comprehensive lead optimization program led to the identification of **DT095895, a small molecule development candidate with a "best-in class" potential**. The *in vitro* and *in vivo* pharmacological characterization of DT095895 drug candidate are presented.



DT095895 is a selective EP_4 receptor antagonist

DT095895 was identified through a comprehensive lead optimization program at Domain Therapeutics. This candidate shows well balanced pharmacodynamic and pharmacokinetic properties, which place it as a best-in-class compound.

- ✓ DT095895 has drug-like properties :
  - Lipinski rules compliant
  - Well balanced lipophilicity : $LogD_{7.4} = 2.0$
  - High Fsp^3 : 0.42, which confer a good "three dimensionality" to the structure
- ✓ **DT095895 is a selective antagonist of the *h*EP4 receptor**
  - nM-potency in a functional assay using our cAMP biosensor : $IC_{50} = 4.2$ nM
  - Antagonist activity on all pathways activated by PGE_2 with similar potencies in our bioSensAll® platform
  - Conserved functional activity across species : $IC_{50} = 11.1$ nM on *m*EP_4 receptor
  - No off-target activity identified in a large panel screen including 80 targets (GPRC and ion channels)
- ✓ **Excellent plasma exposure after oral administration in mouse, rat and dog with excellent bioavailabilities**
- ✓ Not brain penetrant
- ✓ No toxicity flag
  - hERG $IC_{50} > 30$ µM
  - Ames negative @ 100 µM
  - No reversible or time-dependent CYP inhibition : $IC_{50} > 10$ µM on 7 CYP isoforms
  - DT095895 is well tolerated up to 1000 mg/kg in an acute toxicity study in mice
- ✓ DT095895 advantageously compares to clinical competitors in a set of ADME/PK parameters

		DT095895			Grapiprant	E7046	CR6086
On-target activity	*h*EP_4 IC_{50} (nM) [cAMP] / pIC_{50}	4.2 / 8.4			6.3 / 8.2	17 / 7.8	8.0 / 8.1
	LLE	6.0			3.5	3.3	4.9
	pCREB inhibition in Mouse Whole Blood IC_{50} (nM)	38-51			355-907	607-1314	403-5471
Phys. Chem.	MW / TPSA / cLogP	462.5 / 79 / 2.4			491.6 / 114/ 4.7	483.4 / 94 / 4.5	472.5 / 70 / 3.2
	Kinetic solubility (µM)	190			198	> 200	188
ADME	Microsomes : CL_{int} (m/r/h) [µL/min/mg]	27.7 / <9.6 / 10.5			<9.6 / <9.6 / 11.7	<9.6 / <9.6 / <9.6	<9.6 / <9.6 / <9.6
	Caco-2 Papp a>b [10^{-6} cm/s] / efflux	8.2 / 2.0			0.07 / 207	1.5 / 8.0	12.4 / 1.1
PK	Species	Mouse	Rat	Dog	Mouse	Mouse	Mouse
	Dose [mg/kg] (iv / po)	2 / 10	2 / 10	1 / 3	2 / 10	2 / 10	2 / 10
	In vivo Cl [L/h/kg]	0.3	0.095	nc	11.4	2.5	0.3
	$t_{1/2}$ [h] (iv / po)	8.35 / 8.00	5.7 / 5.0	8.2 / 6.6	1.30 / 5.98	nc	5.61 / 6.91
	$AUC_{0\text{-}24h}$ (ng.h/mL) (iv / po)	5843 / 15991	20239 / 68000	38653 / 90142	175 / 301	797 / 2333	7390 / 27238
	Vss [L/kg]	4.1	0.8	nc	21	nc	2.1
	C_{max} [ng/mL] (po)	4812	4807	18582	113	736	2649
	F [%]	42	67	78	32	59	77

Note: PK experiments were performed using 5% DMSO / 95% (10% HP-β-CD on water) as a vehicle, nc : not calculated. Grapiprant was bought at Interchim Bioscience, Reference : AXSHJ2. E7046 was resynthesized according to Albu *et al. Oncoimmunology*, **2017**, e1338239. CR6086 was resynthesized according to Caselli *et al. Arthritis Research & Therapy*, **2018**, 20.

DT095895 induces a superior target engagement

As the EP_4 receptor is primary coupled to the $G_{\alpha S}$ protein, its activation in immune cells leads to elevated intracellular cAMP levels and subsequent phosphorylation of the transcription factor CREB. DT095895 target engagement was assessed in phospho-flow assays in whole blood to demonstrate its capacity to inhibit an EP_4R agonist-induced CREB phosphorylation in an immune cell subset and to compare to clinical competitors. Such assays will be later used for the development of target engagement biomarkers to support Phase I studies.

Murine Whole blood

DT095895 inhibits the EP_4R agonist-induced CREB phosphorylation in $CD45^+$, $CD3^+$, $CD4^+$ and $CD8^+$ cells in Mouse Whole Blood with IC_{50} in the 38-51 nM range and induces superior target engagement when compared to clinical competitors.

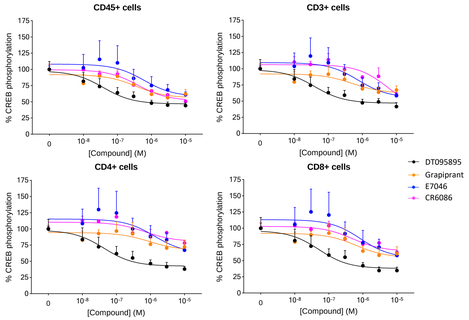


Fig. 1 & Table 1 : Whole blood was collected from mice via retro-orbital bleeding in EDTA tubes and pooled. Whole blood samples were treated *ex vivo* with an EP_4R agonist at 8 doses for 10 min, followed by a treatment with an EP_4R agonist and incubation for 30 min. Each experiment was performed in triplicate. At the end of the incubation period, cells were fixed, and samples were processed for immunofluorescence staining and flow cytometry detection according to the following panel : CD45/CD3/CD4/CD8/pCREB/Viability marker.

EP_4R antagonist	IC_{50} (nM)			
	$CD45^+$	$CD3^+$	$CD4^+$	$CD8^+$
DT095895	38	39	43	51
Grapiprant	355	518	907	634
E7046	607	751	1314	859
CR6086	403	5471	716	503

Human Whole blood

DT095895 inhibits efficiently the EP_4R agonist-induced CREB phosphorylation in $CD45^+$, $CD3^+$, $CD4^+$ and $CD8^+$ cells in Human Whole Blood at a lower concentration than clinical competitors.

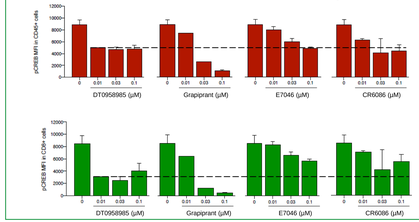


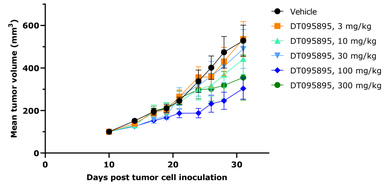
Fig. 2 : Whole blood from one healthy donor was collected in EDTA tubes. Whole blood samples were treated *ex vivo* with an EP_4R antagonist at 3 doses for 10 min, followed by a treatment with an EP_4R agonist and incubation for 15 min. Each experiment was performed in triplicate. At the end of the incubation period, cells were fixed, and samples were processed for immunofluorescence staining and flow cytometry detection according to the following panel : CD45/CD3/CD4/CD8/pCREB/Viability marker. $CD3^+$ and $CD4^+$ data not shown. These preliminary data will be completed with full Concentration-Response curve experiments.

DT095895 induces strong Tumor Growth Inhibition in syngeneic mouse models

Anti-tumor activity of DT095895 after oral administration was evaluated in syngeneic mouse tumor models model, in monotherapy and in combination with an immune checkpoint inhibitor.

Pan02

DT095895 induces 42.6% tumor growth inhibition after oral administration @ 100 mg/kg in monotherapy in a Pan02 pancreatic cancer model.



Treatment	Mean tumor volume (mm^3)[a] on Day 31	TGI (%)	*P* value [b]
Vehicle	528.6 ± 73.0	-	-
DT095895, 3 mg/kg	536.2 ± 82.5	-1.4	ns
DT095895, 10 mg/kg	442.8 ± 72.5	16.2	ns
DT095895, 30 mg/kg	489.4 ± 91.2	7.4	ns
DT095895, 100 mg/kg	303.5 ± 49.7	42.6	< 0.05
DT095895, 300 mg/kg	355.3 ±106.3	32.8	ns

Note: a. Mean ± SEM; b. compared with vehicle group tumor volume on day 31 using t test method; ns : non significative

Fig. 3 & Table 2 : 10 mice /group. DT095895 treatment started once mean tumor volumes reached approximatively 100 mm^3, mice were treated po, QD, over a 21-day period. Mean tumor volumes are presented.

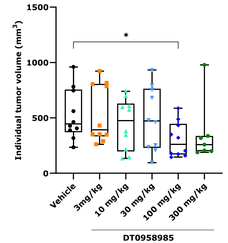
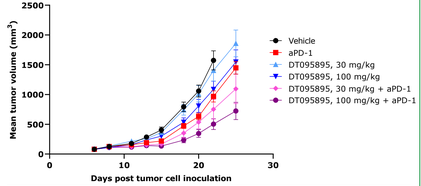


Fig. 5 : Box plot representation of individual tumor volumes on Day 31. Median tumor volumes and quartiles are presented.

MCA205

DT095895 enhances aPD-1 activity and induces 68.0% tumor growth inhibition after oral administration @ 100 mg/kg in combination with aPD-1 in a MCA205 sarcoma model.



Treatment	Mean tumor volume (mm^3)[a] on Day 22	TGI (%)	*P* value [b]	*P* value [c]
Vehicle	1573.4 ± 159.9	-	-	-
DT095895, 30 mg/kg	1404.1 ± 179.6	10.8	ns	-
DT095895, 100 mg/kg	1085.3 ± 139.1	31.0	ns	-
aPD-1	968.5 ± 94.3	38.4	< 0.05	-
DT095895, 30 mg/kg + aPD-1	751.6 ± 164.1	52.2	< 0.001	ns
DT095895, 100 mg/kg + a-PD-1	502.1± 91.4	68.0	< 0.0001	< 0.05

Note: a. Mean ± SEM; b. compared with vehicle group tumor volume on day 22 using t test method; c. compared with aPD-1 group tumor volume on day 22 using t test method; ns : non significative

Fig. 4 & Table 3 : 10 mice /group. DT095895 treatment started once mean tumor volumes reached approximatively 80 mm^3, mice were treated po, QD, over a 20-day period. aPD-1 was administered ip on days 6, 9, 12 and 15 (5 mg/kg). Mean tumor volumes are presented.

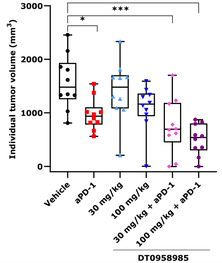


Fig 6 : Box plot representation of individual tumor volumes on Day 22. Median tumor volumes and quartiles are presented.

CONCLUSION

DT095895 is a selective EP_4R antagonist development candidate with drug-like properties and favorable PK parameters. DT095895 efficiently engages the EP_4 receptor in mouse and human whole blood and demonstrates strong anti-tumor effects in syngeneic mouse tumor models, both as a monotherapy and in combination with ICI. DT095895 positions itself as a promising best-in-class drug candidate able to inhibit the PGE_2-induced immunosuppression in solid tumors and to restore tumor immunity. DT095895 progresses in regulatory development.

For any question or interest, please contact : sschann@domaintherapeutics.com (Science); msidhoum@domaintherapeutics.com (BD)