

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Application of:	DIX <i>et al.</i>	Art Unit:	1647
Serial No:	12/835,065	Docket No:	4030B
Filed:	07/13/2010	Customer No:	26693
For:	VEGF Antagonist Formulations	Examiner:	STOICA, Elly Gerald

DECLARATION PURUANT TO 37 C.F.R. § 1.131**OF DANIEL B. DIX, KELLY FRYE, AND SUSAN KAUTZ**

1. We, Daniel B. Dix, Susan Kautz, and Kelly Frye, do declare and state:
2. We are co-inventors of claims 1, 3, 4, 6, 8, and 25 of the above-identified application.
3. Prior to November 23, 2004, we completed our invention as described and claimed in the above-referenced application, as evidenced by the following documents developed or executed under our direction:
 - a. EXHIBIT A: Regeneron Notebook No. 4143, pages 60 - 61, for project VEGF-SS014, signed and dated November 21, 2000. These pages disclose the manufacture of SS014 VEGF Trap Formulation 1, which provides 25 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 5% sucrose, 50 mM NaCl, and 0.1% polysorbate-20.
 - b. EXHIBIT B: Stability study of SS014 VEGF Trap Formulation 1, signed and dated on November 21, 2000, November 22, 2000, November 27, 2000, and

December 5, 2000. Discloses visual inspection results of Formulation 1 subjected to vortex stress, freeze/thaw stress, and temperature stress.

c. EXHIBIT C: Regeneron Notebook No. 4212, page 50, for project VEGF-SS017, signed and dated January 30, 2001. This page discloses the manufacture of two formulations of VEGF Trap lot # VGT01001T: (a) 35.4 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 100 mM NaCl, and 0.04% polysorbate-20, pH 6.17; and (b) 24.3 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 20% sucrose, and 0.1% polysorbate-20, pH 6.05.

d. EXHIBIT D: Regeneron Notebook No. 4216, page 50, for project VEGF-SS017, signed and dated February 28, 2001. This page discloses the 37°C stability analysis data for the VEGF SS017 formulation #1: 25 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 20% sucrose, and 0.1% polysorbate-20, pH 6.0.

e. EXHIBIT E: Data sheet disclosing stability study of VEGF Trap formulation VGFT-SS065 (50 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 20% sucrose, and 0.1% polysorbate-20, pH 6.00) by analyzing OD 405 nm. Of particular note is the entry for the 5°C six month study results, which are signed and dated on May 24, 2004.

f. EXHIBIT F: Data sheet disclosing stability study of VEGF Trap formulation VGFT-SS065 (50 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 20% sucrose, and 0.1% polysorbate-20, pH 6.00) by visual inspection analysis. Of particular note is the entry for the 5°C six month study results, which are signed and dated on May 24, 2004.

g. EXHIBIT G: Data sheet disclosing stability study of VEGF Trap formulation VGFT-SS065 (50 mg/mL VEGF Trap protein, 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 20% sucrose, and 0.1% polysorbate-20, pH 6.00) by analyzing pH. Of particular note is the entry for the 5°C six month study results, which are signed and dated on May 24, 2004.

4. At the time of the invention, I was an employee of Regeneron Pharmaceuticals, Inc., to which the above referenced application is assigned.

5. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Nov. 10, 2011Daniel B. Dix
Daniel DixDate: Nov. 10, 2011Susan Kautz
Susan KautzDate: Nov. 17, 2011Kelly Frye
Kelly Frye

EXHIBIT A

60

PROJECT VEGF - 55014

Notebook No. 443

Continued From Page 59

Calculations

① Protein 56.09 mg/mL
 $x (56.09 \text{ mg/mL}) = 1.75 \text{ mL} (25 \text{ mg/mL})$
 $\Rightarrow x = 0.7806 \text{ mL}$

② Buffer (using 100 mM Phos + Citrate stocks @ pH 6.0)
 In bulk there is $3.903 \mu\text{mol}$ of each buffer component; need $8.75 \mu\text{mol}$ of each
 $8.75 \mu\text{mol} - 3.903 \mu\text{mol} = 4.847 \mu\text{mol} \div 100 \frac{\mu\text{mol}}{\text{mL}} = 0.04847 \text{ mL}$ of each 100 mM Phos & 100 mM Cit. pH 6.0

③ NaCl (1.5 M stock)
 F1:
 $x (1.5 \text{ M}) = 1.75 \text{ mL} (0.050 \text{ M})$
 $\Rightarrow x = 0.0583 \text{ mL}$

F2:
 $x (1.5 \text{ M}) = 1.75 \text{ mL} (0.100 \text{ M})$
 $\Rightarrow x = 0.1167 \text{ mL}$

Dextrose

F1:
 $\frac{5}{100} \times 1.75 \text{ mL} = 0.0875 \text{ g}$

F2:
 $\frac{20}{100} \times 1.75 \text{ mL} = 0.350 \text{ g}$

⑤ Polysorbate - 20 (10% stock)

$\frac{0.1}{100} \times 1.75 \times 10 = 0.0175 \text{ mL}$

Density

F1 = 1.0183

F2 = 1.081

Total wt

F1 = 1.782 g

F2 = 1.892 g

WFI

F1 = 0.7411 g

F2 = 0.5469 g

Continued on Page 061

Read and Understood By

Rh Zhan

Signed

4/24/00

Date

Harold J

Signed

9/20/03

Date

Preparation	Company	Lot #	Formulation 1:		Formulation 2:	
			Lot # 200 - 303		Lot # 200 - 304	
Reagents	Company	Lot #	Theoretical	Actual	Theoretical	Actual
WFI	Regeneron	112000	0.7411g	0.50g	0.5489g	0.50g
100mM Phos pH 6	—	RL-443-50-02	0.0485g	0.0489g	0.0485g	0.0489g
			Mix	✓	Mix	✓
100mM Cit pH 6	—	RL-443-53-01	0.0485g	0.0497g	0.0485g	0.0491g
			Mix	✓	Mix	✓
Sucrose	Mallinkrodt	7723 T08 H34	0.0875g	0.0882g	0.350g	0.3505g
			Mix	✓	Mix	✓
Polyethylene glycol (10%)	—	RL-443-46	0.075g	0.0181g	0.075g	0.0194g
			Mix	✓	Mix	✓
NaCl (1.5M)	—	RL-443-11-03	0.0583g	0.0591g	0.0167g	0.01201g
			Mix	✓	Mix	✓
VEGF Trp (5605mg/ml)	Regeneron	001025	0.7806g	0.7808g	0.7806g	0.7809g
			Mix	✓	Mix	✓
pH	—	—	6	6.02	6	5.95
				no adjustment		no adj
Total vol	—	—	1.782g	1.78g	1.872g	1.87g

* f = C + Vx analyzed immediately; incubation start @ 18:45h
 F/T put into -80°C @ 18:45h (11/21/00)

Continued on Page —

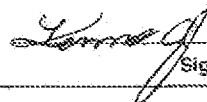
Read and Understood By



Signed

11/21/00

Date



Signed

9/29/00

Date

EXHIBIT B

Stability Study VEGF-SS014 Analysis of VEGF Trap by Visual Inspection

Stress Test	Length of Stress Test	Formulation 1	Formulation 2	Initials and Date
		5mM Phos/5mM Cit pH 6.0 5% Sucrose 50 mM NaCl 0.1% Twn-20 25 mg/mL	5mM Phos/5mM Cit pH 6.0 20% Sucrose 100 mM NaCl 0.1% Twn-20 25 mg/mL	
Vortex (min)	0	clear, no ppt	clear, no ppt	KZ 11/21/00
	15	clear, no ppt	clear, no ppt	KZ 11/21/00
	60	clear, no ppt	clear, no ppt	KZ 11/21/00
Freeze/Thaw (Cycles)	0	clear, no ppt	clear, no ppt	KZ 11/21/00
	6	clear, no ppt	clear, no ppt	KZ 11/22/00
37°C (days)	0	clear, no ppt	clear, no ppt	KZ 11/21/00
	6.00	clear, no ppt	clear, no ppt	KZ 11/27/00
	12.00	clear, no ppt	clear, no ppt	KZ 12/05/00

EXHIBIT C

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PROJECT Receipt of Protein

Notebook No. 4212

Continued From Page

Received from the Process Development Group the following:

# of Vials	Type of Vessel	Vol/vial	VEGF-Trap	lot#
8	50 ml PC tube	20 ml	35.4 mg/ml	VGTO1001T
2	50 ml PP tube	20 ml	24.3 mg/ml	VGTF01001T
2	5 ml PC tube	2.5 ml	24.3 mg/ml	VGTF01001T

Composition:

lot# VGTO1001T (35.4 mg/ml VEGF-Trap)
 5 mM Phosphate
 5 mM Citrate
 100 mM NaCl
 0.04% Polysorbate 20
 pH 6.17

lot# VGTF01001T (24.3 mg/ml VEGF-Trap)
 5 mM Phosphate
 5 mM Citrate
 100 mM NaCl
 20% Sucrose
 0.1% Polysorbate 20
 pH 6.05

The VEGF Trap Dry Substance (lot# VGTO1001T) aliquot into 50 ml volume in 15 ml Falcon tube and frozen @ -80°C.

The Drug Product (lot# VGTF01001T) frozen @ -80°C as received.

Continued on Page

Read and Understood By

B. Poliheman
 Signed

01/30/01
 Date

Eric Rife
 Signed

2/15/02
 Date

EXHIBIT D

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PROJECT VEGF-SS017

Notebook No. 4216

Continued From Page 70

Summary VEGF-SS017 37°C

Stability Study VEGF-SS017 Analysis of VEGF TRAP (25 mg/mL) by SEC-HPLC When Incubated at 37°C

Formulation	Length of Incub. (days)	HMW Peak Area (24'-55')	Native Peak Area (40')	LMW Peak Area (55')	LMW Peak Area (65')	Increase in Area of Late Shoulder?	[VEGF-TRAP] mg/ml	% VEGF-TRAP Recovered	% VEGF-TRAP Degraded
#1:	0	216	102848	0	318	-	24.58	100	0.21
5 mM Phos/5 mM Cit	0	209	102896	0	320	-	24.59	100	0.20
pH 6.0	0	200	102421	0	300	-	24.52	100	0.19
100 mM NaCl	0	189	102438	0	318	-	24.53	100	0.18
0.1% Polysorbate 20	1.0	257	100094	0	309	-	23.98	98	0.26
20% Sucrose	1.0	253	100999	0	308	-	24.20	99	0.25
25 mg/mL VEGF Trap	2.0	310	94992	0	288	-	22.76	93	0.33
	2.0	258	94255	0	292	-	22.57	92	0.27
	3.1	380	101453	0	310	-	24.39	99	0.37
	3.1	373	101518	0	312	-	24.35	99	0.37
	7.1	451	95404	0	290	-	22.89	93	0.47
	7.1	449	95256	0	292	-	22.88	93	0.47
	10.0	549	99738	0	314	-	23.96	98	0.55
	10.0	544	100042	0	315	-	24.03	98	0.54
	14.1	656	97388	55	306	-	23.43	95	0.72
	14.1	640	96978	126	412	-	23.36	95	0.76
	17.0	781	102885	83	326	-	24.80	101	0.83
	17.0	752	102368	14	334	-	24.55	100	0.74
	21.0	956	95980	471	381	-	23.27	95	1.46
	21.0	846	95498	94	308	-	23.04	94	0.97

Data From Sequence #022018 #0221018

Time (days)	%HMW Species	%Native	%LMW Species
0	0.210	99.79	0
0	0.203	99.80	0
0	0.195	99.81	0
0	0.184	99.82	0
1.0	0.256	99.74	0
1.0	0.260	99.74	0
2.0	0.325	99.57	0
2.0	0.273	99.73	0
3.1	0.373	99.63	0
3.1	0.366	99.63	0
7.1	0.471	99.53	0
7.1	0.469	99.53	0
10.0	0.547	99.45	0
10.0	0.541	99.46	0
14.1	0.669	99.28	0.06
14.1	0.655	99.22	0.13
17.0	0.753	99.17	0.08
17.0	0.729	99.26	0.01
21.0	0.981	98.54	0.48
21.0	0.877	98.08	0.10

From a 1st order plot
a rate constant of
 $3.56 \times 10^{-4} \text{ day}^{-1}$
obtained.

Continued on Page 75

Read and Understood By

Gjorlchara
Signed

2/28/01
Date

Erica Ryfus
Signed

2/15/02
Date

EXHIBIT E

Stability Study VGFT-SS065 Analysis of VEGF Trap by OD 405 nm

Stress Test	Time Point	Formulation 1	Formulation 2	Formulation 3	Initials and Date
		5 mM Phosphate, 5 mM Citrate, 100 mM NaCl, 0.1% Polysorbate20, 20 % Sucrose, 50 mg/mL VEGF, pH 6.00	10mM Phosphate, 50 mM NaCl, 0.1% Polysorbate20, 5% Sucrose, 50 mg/mL VEGF, pH 6.25	10mM Phosphate, 50 mM NaCl, 0.1% Polysorbate 20, 20% Sucrose, 50 mg/mL VEGF, pH 6.25	
t=0	0				
Vortex	60 minutes	0.059	0.060	0.060	AJ 12-5-03
	120 minutes	0.075	0.075	0.069	AJ 12-5-03
Freeze/Thaw	8 cycles	0.050	0.053	0.057	AJ 12-5-03
45°C	7 days	0.054	0.054	0.054	AJ 12-4-03
	14 days	0.055	0.062	0.059	AJ 12-10-03
	28 days	0.043	0.050	0.046	AJ 3-3-04
25°C	3 months	0.045	0.047	0.046	AJ 2-24-04
	6 months	0.051	0.054	0.056	AJ 5-24-04
	9 months	~~0.010~~ 0.046	~~0.013~~ 0.048	~~0.012~~ 0.048	AJ 8-23-04
	12 months	0.052	0.058	0.054	AJ 11-19-04
	18 months	0.049	0.052	0.051	AJ 5-20-05
*	24 months	0.050	0.051	0.051	JD 11-15-05
5°C	3 months	0.045	0.047	0.044	AJ 2-24-04
	6 months	~~0.052~~ 0.052	0.055	0.052	AJ 5-24-04
	9 months	~~0.011~~ 0.045	~~0.013~~ 0.049	~~0.013~~ 0.049	AJ 8-23-04
	12 months	0.052	0.056	0.052	AJ 11-19-04
	18 months	0.048	0.050	0.047	AJ 5-20-05
*	24 months	0.048	0.051	0.047	JD 11-15-05

* volumes slightly less than 150 uL JD 11/15/05

EXHIBIT F

Stability Study VGFT-SS065 Analysis of VEGF Trap by Visual Inspection

Stress Test	Time Point	Formulation 1	Formulation 2	Formulation 3	Initials and Date
5°C	1 month				
	2 months	cnp	cnp	cnp	N 1-26-04
	3 months	cnp	cnp	cnp	N 2-24-04
	4 months	cnp	cnp	cnp	N 3-28-04
	5 months	cnp	cnp	cnp	N 4-28-04
	6 months	cnp	cnp	cnp	N 5-24-04
	7 months	cnp	cnp	cnp	N 6-23-04
	8 months	cnp 10mth ppt? * = FUZZ (9mth)	cnp * = FUZZ (9mth)	cnp	SMZ 7/22/04
	9 months	cnp cant find spec!	cnp clear FUZZ	cnp	N 8-23-04
	10 months	cnp 10mth ppt? FUZZ 9mth	cnp 4mth FUZZ	cnp	SMZ 9/21/04
	11 months	cnp	cnp	cnp 18m spec	
	12 months	cnp	clear 1 particle	cnp	SMZ 11/19/04
	13 months	cnp	cnp	cnp	N 12/11/04
	14 months	cnp	cnp except 24m	cnp	N 1-20-05
	15 months	cnp	fluzz? 24m	cnp	SMZ 2/18/05
	16 months	cnp	TBO - white filament + 24m - white ppt all else cnp	18m 2x white fiber all else cnp	JC 3/21/05
	17 months	cnp	24m white ppt all else cnp	18m 2 fibers all else cnp	SMZ 4/19/05
	18 months	All clear, no TBO particles 18m particulate + 24m particulate	All clear, TBO + TBO A = particles TBO B = 1 long filament 24m particulate	All clear 18m of filament ppt all else ppt	JC 5/20/05
	19 months	cnp	all else cnp	cnp	SMZ 6/16/05
	20 months	24m = clear, 1 ppt. TBO = clear, 1 ppt.	24m clear, 1 ppt. TBO A clear, 1 sm ppt. TBO B clear, 1 sm filament	24m = clear, many filamentous ppt. TBO nothing ppt (A+B)	JC 7/18/05
	21 months	24m clear 1 ppt - TBO clear, 1 clear particulate	24m clear 1 ppt TBO A clear 1 sm ppt TBO B clear 1 sm + 1 sm filament	24m clear 3-4 filament cnp TBO A 2-3 ppt. TBO B 3 ppt.	JC 8/15/05
	22 months	24m clear, 1 ppt TBO clear no ppt.	24m clear 1 sm ppt TBO A = 1 sm + 1 sm TBO B = 1 sm + 1 sm TBO C = 1 sm + 1 sm	24m clear, 1 sm ppt TBO A + B 1 very sm particle in each	JC 9/19/05
	23 months	24m + TBO = cnp.	As per 22mth Vis. 22	24m clear, 1 sm particle TBO clear 1 sm ppt TBO B = 1 sm + 1 sm TBO C = 1 sm + 1 sm	JC 10/17/05
	24 months	TBO = cnp 24m = cnp	TBO A = 1 sm + 1 sm TBO B = 1 sm + 1 sm 24m = 1 sm + 1 sm particle	TBO A = cnp TBO B = 1 v tiny particle 24m = cnp	JD 11/15/05

ppf may be *
dried simulation
washing of top of vial
bt always
mth visits
inside the
overed.

18mth + 24mth
clear, white particles
JC 5/20/05.

EXHIBIT G

Stability Study VGFT-SS065 Analysis of VEGF Trap by pH

Stress Test	Time Point	Formulation 1	Formulation 2	Formulation 3	Initials and Date
		5 mM Phosphate, 5 mM Citrate, 100 mM NaCl, 0.1% Polysorbate20, 20 % Sucrose, 50 mg/mL VEGF, pH 6.00	10mM Phosphate, 50 mM NaCl, 0.1% Polysorbate20, 5% Sucrose, 50 mg/mL VEGF, pH 6.25	10mM Phosphate, 50 mM NaCl, 0.1% Polysorbate 20, 20% Sucrose, 50 mg/mL VEGF, pH 6.25	
t=0	0	6.04	6.24	6.23	VG 11-26-03
45°C	7 days	~~6.2~~ 6.13	~~6.24~~ 6.28	6.27	N 12-1-03
	14 days	6.12	6.31	6.29	N 12-10-03
	28 days	6.20	6.26	6.30	N 2-3-04
25°C	3 months	6.17	6.37	6.33	N 2-24-04
	6 months	6.04	6.22	6.22	N 5-24-04
	9 months	6.05	6.22	6.20	N 8-23-04
	12 months	6.11	6.28	6.24	DMB 11/19/04
	18 months	6.12	6.29	6.24	JC 5/20/05
	24 months	6.10	6.27	6.21	JD 11/15/05
5°C	3 months	5.97	6.18	~~6.23~~ 6.19	N 2-24-04
	6 months	6.09	6.28	6.22	N 5-24-04
	9 months	6.10	6.28	6.26	N 8-23-04
	12 months	6.10	6.30	6.25	DMB 11/19/04
	18 months	6.12	6.31	6.25	JC 5/20/05
	24 months	6.11	6.29	6.24	JD 11/15/05