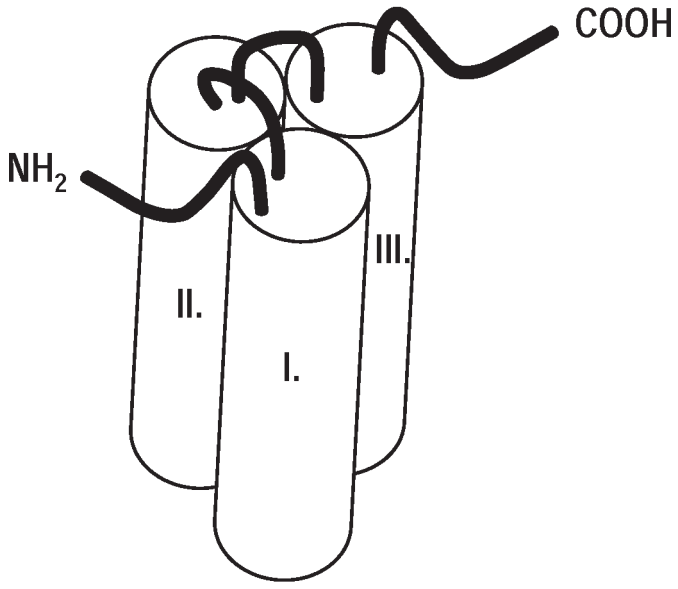
Fig. 1

A.



B.

Fig. 2

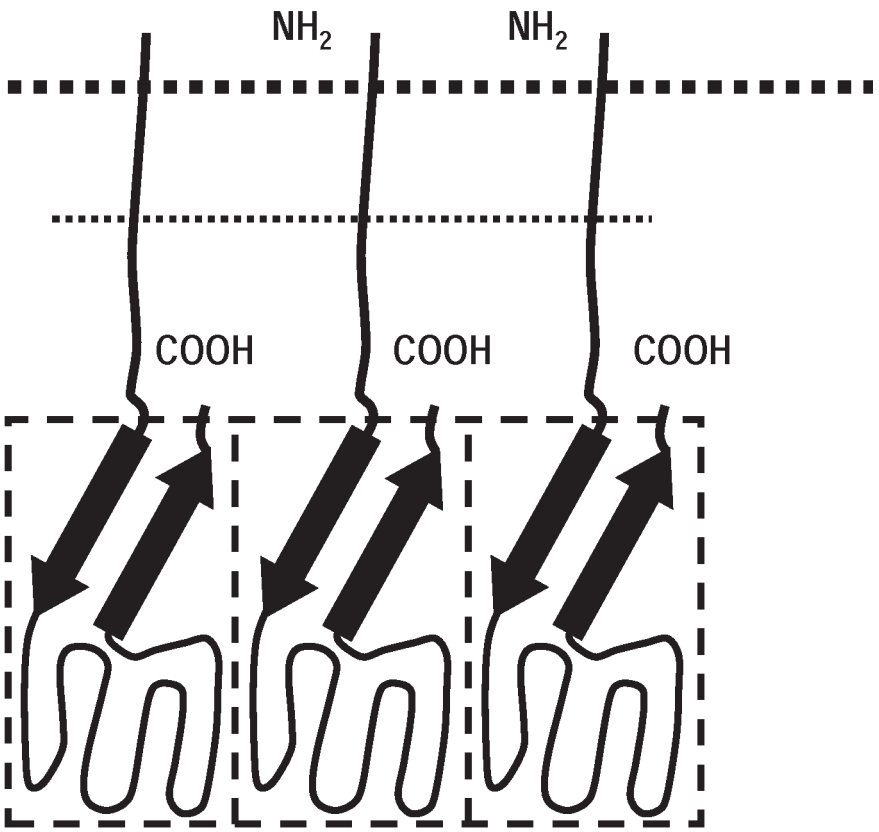


Fig. 3

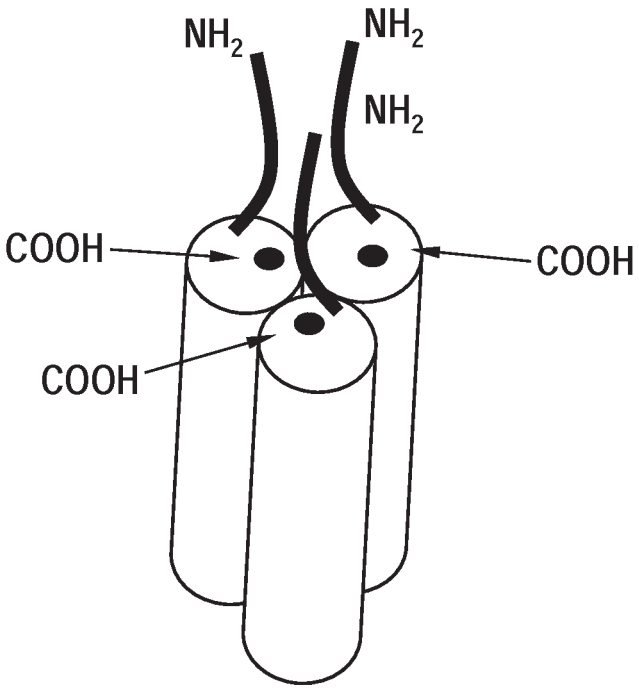


Fig. 4

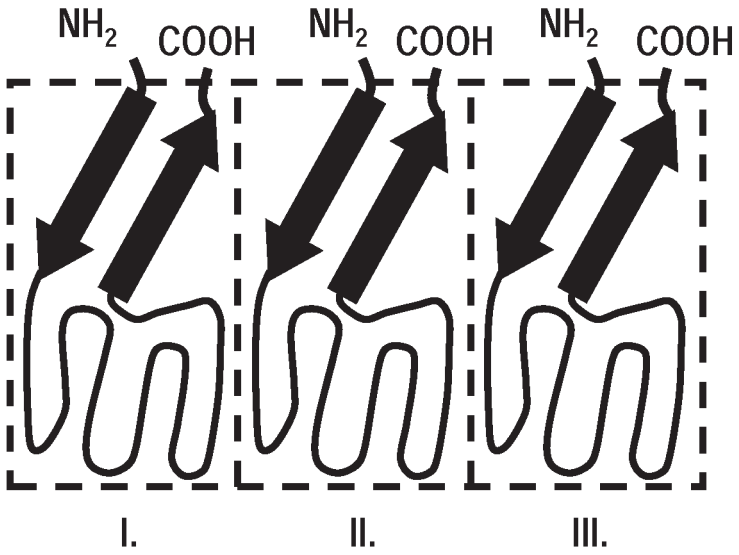


Fig. 5

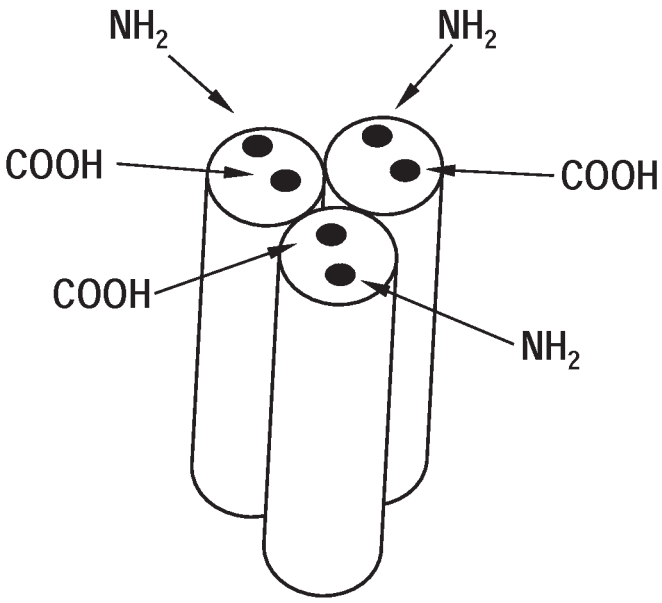


Fig. 6

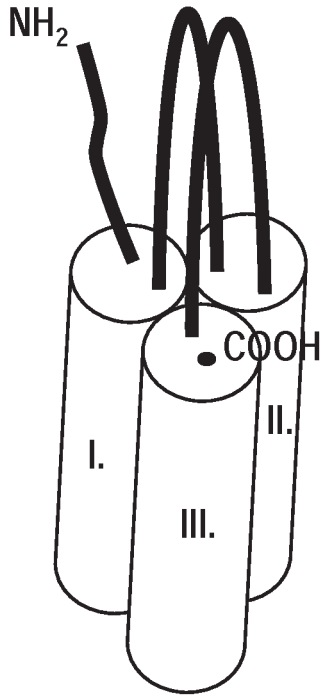


Fig. 7

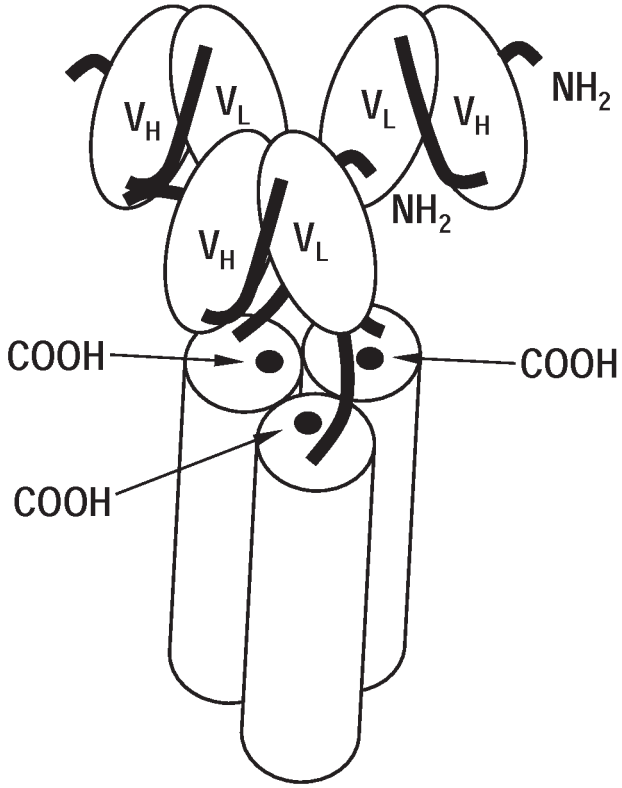


Fig. 8

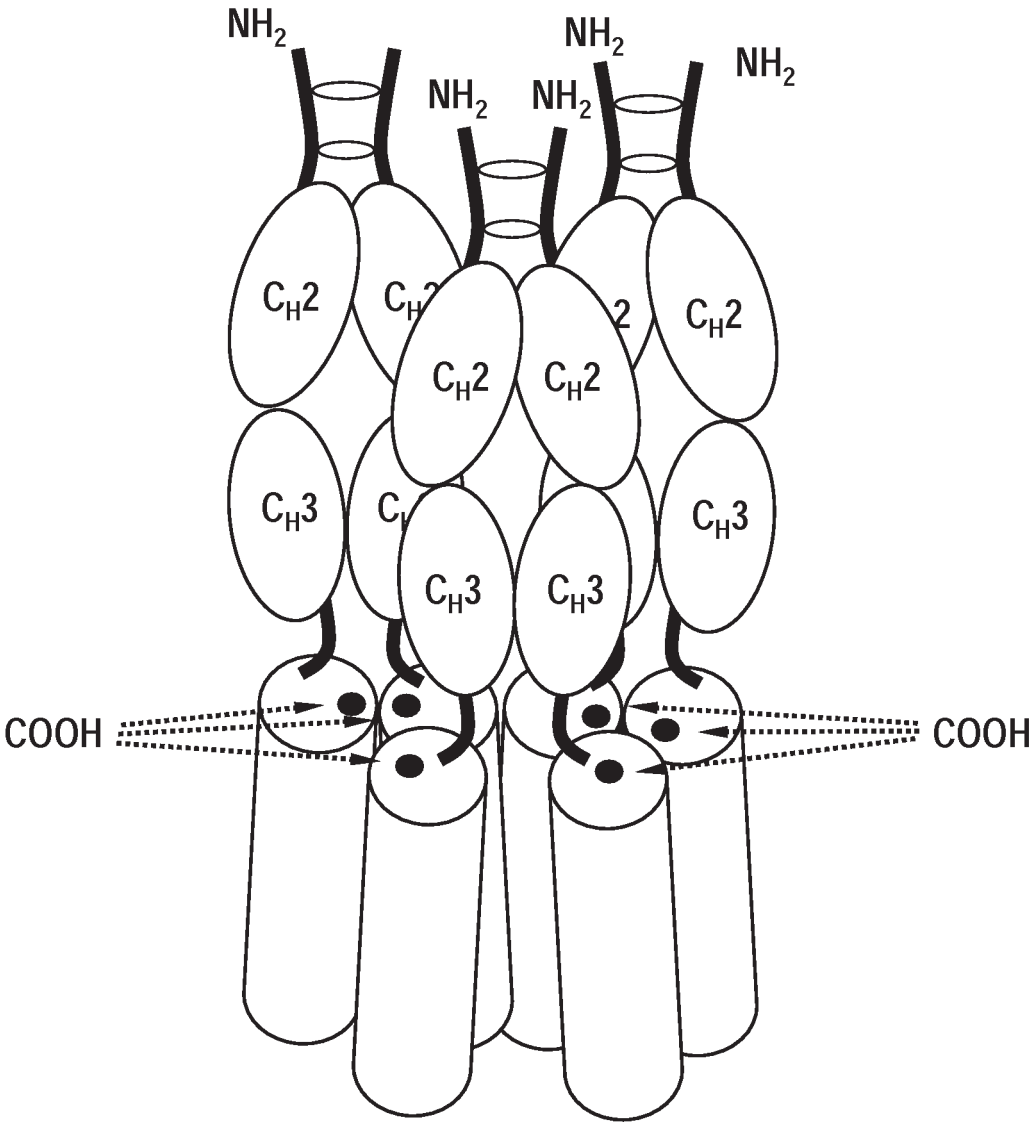
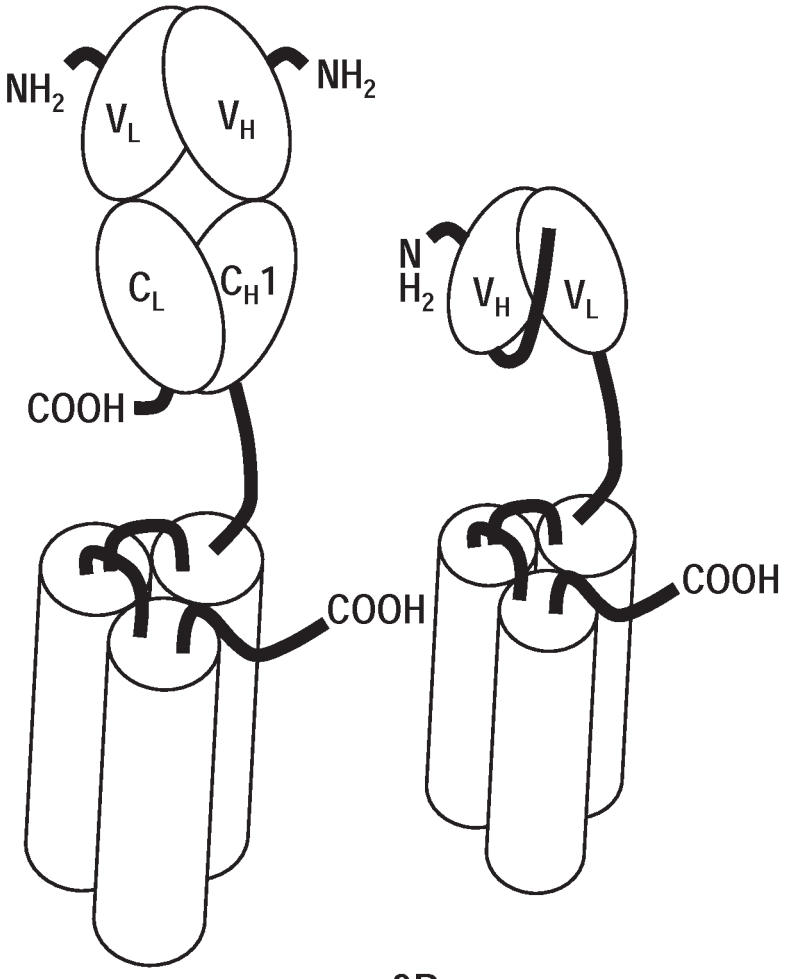

NH2
NH2
NH2
NH2
NH2
CH2
CH2
CH2
CH2
CH2
CH3
CH3
CH3
CH3
COOH
COOH

Fig. 9



9A 9B

Fig. 10

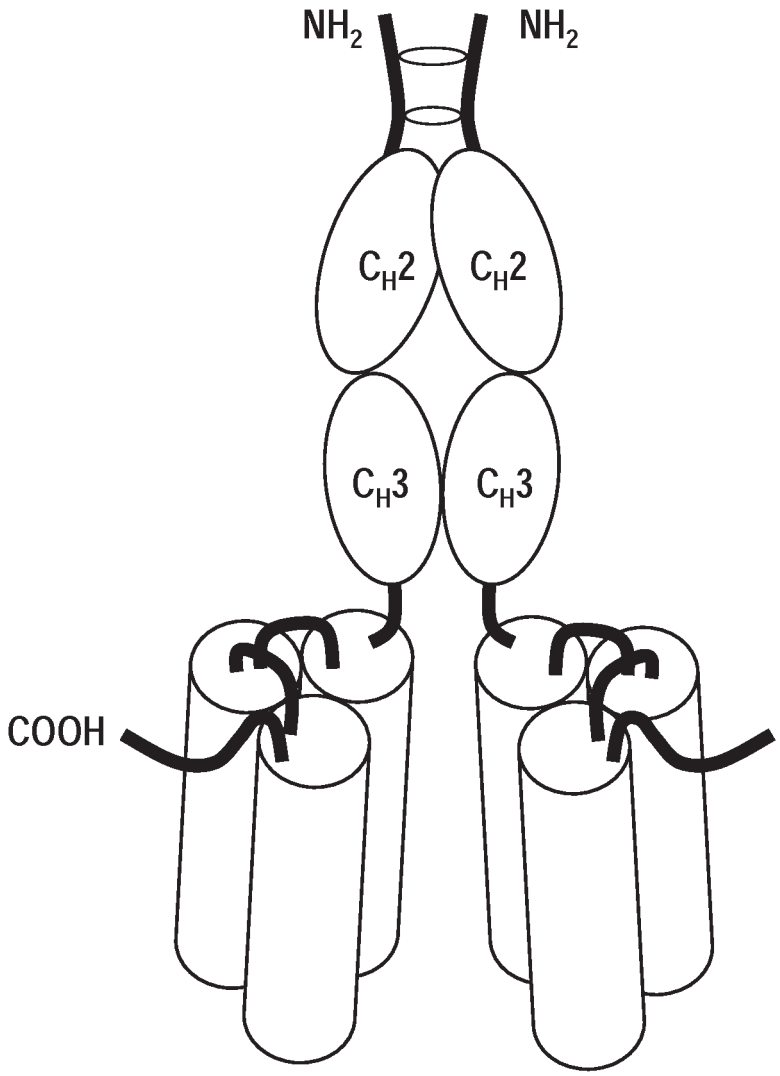


Fig. 11

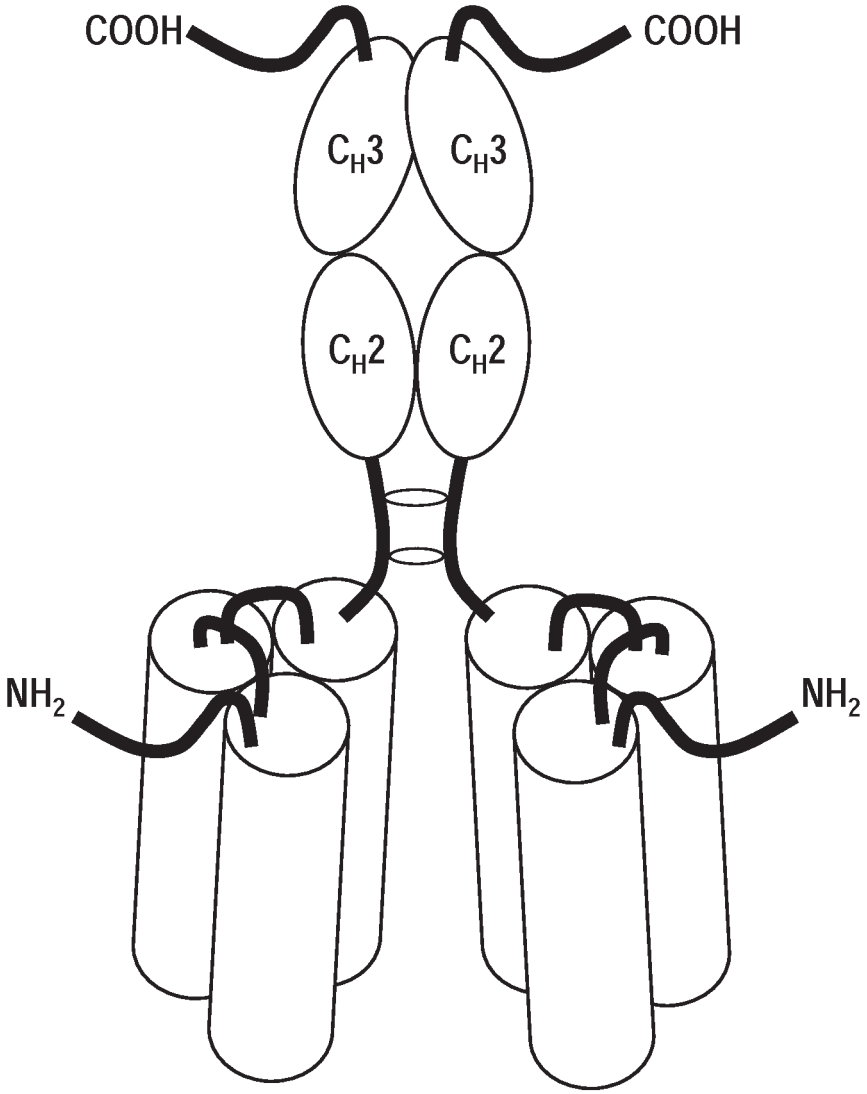


Fig. 12

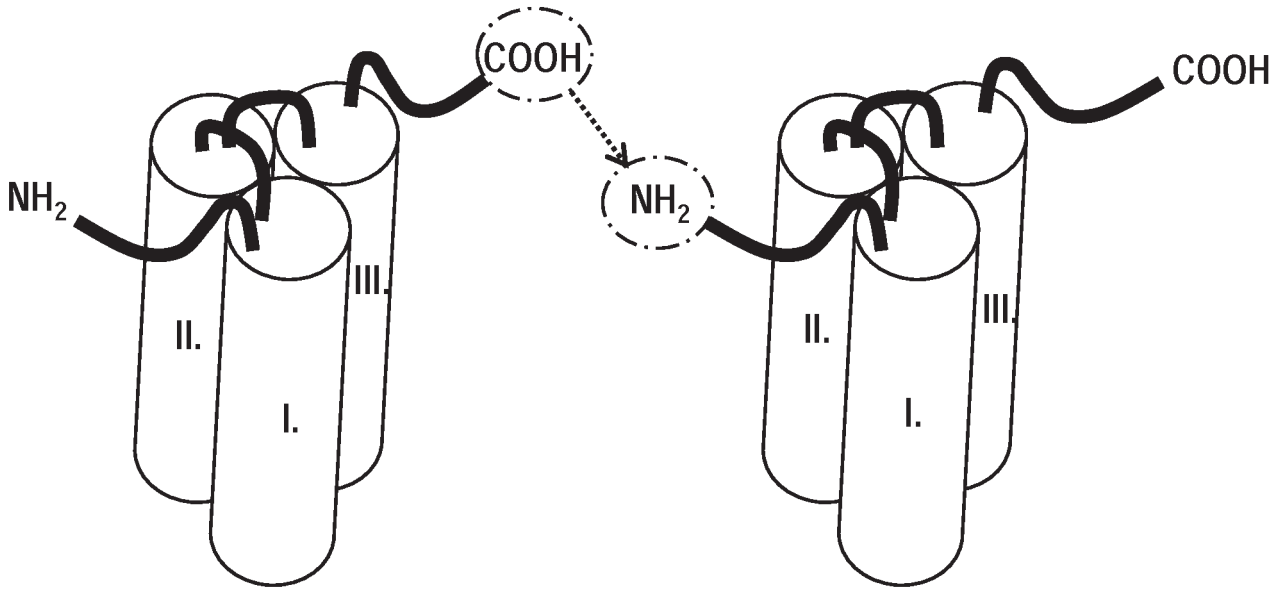


Fig. 13

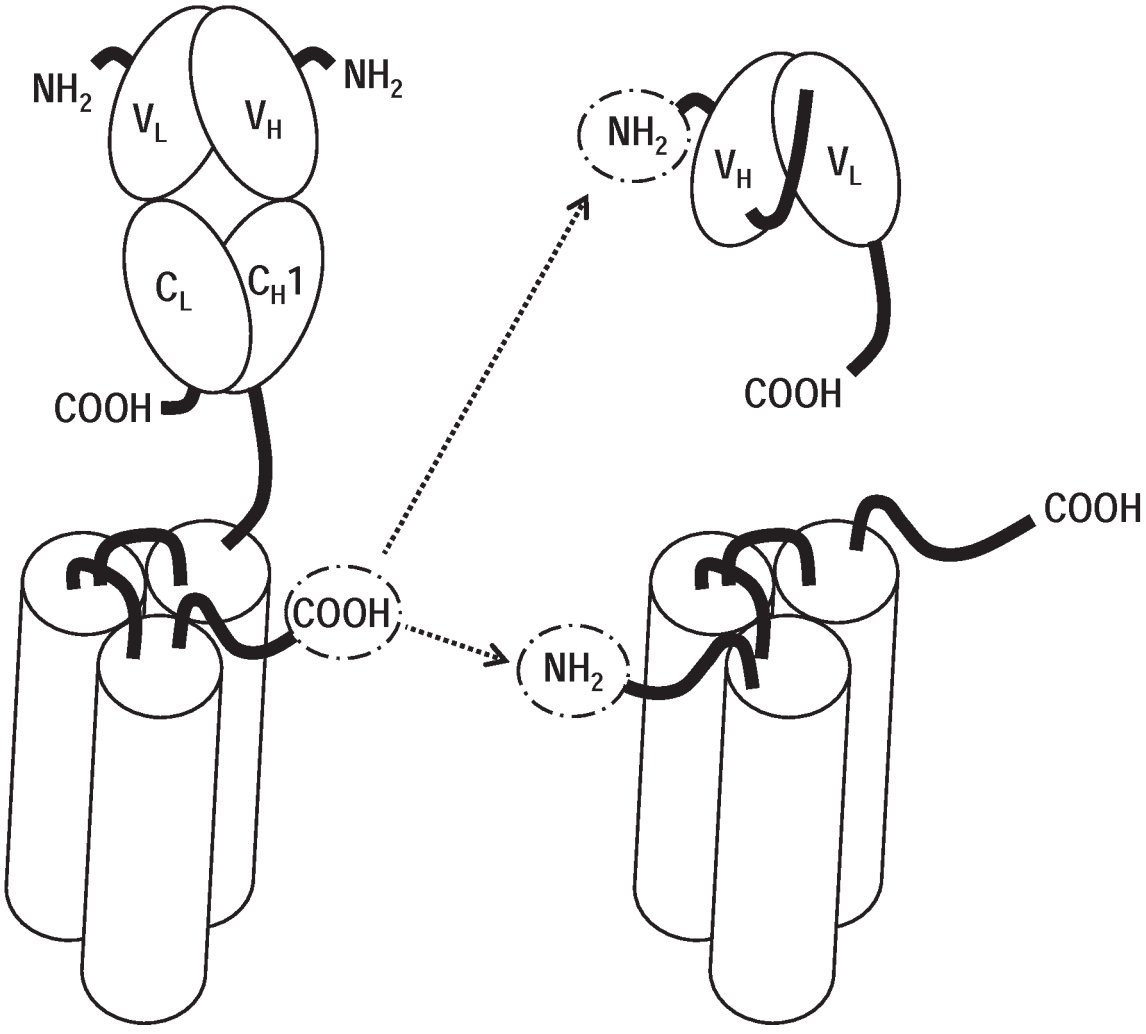


Fig. 14

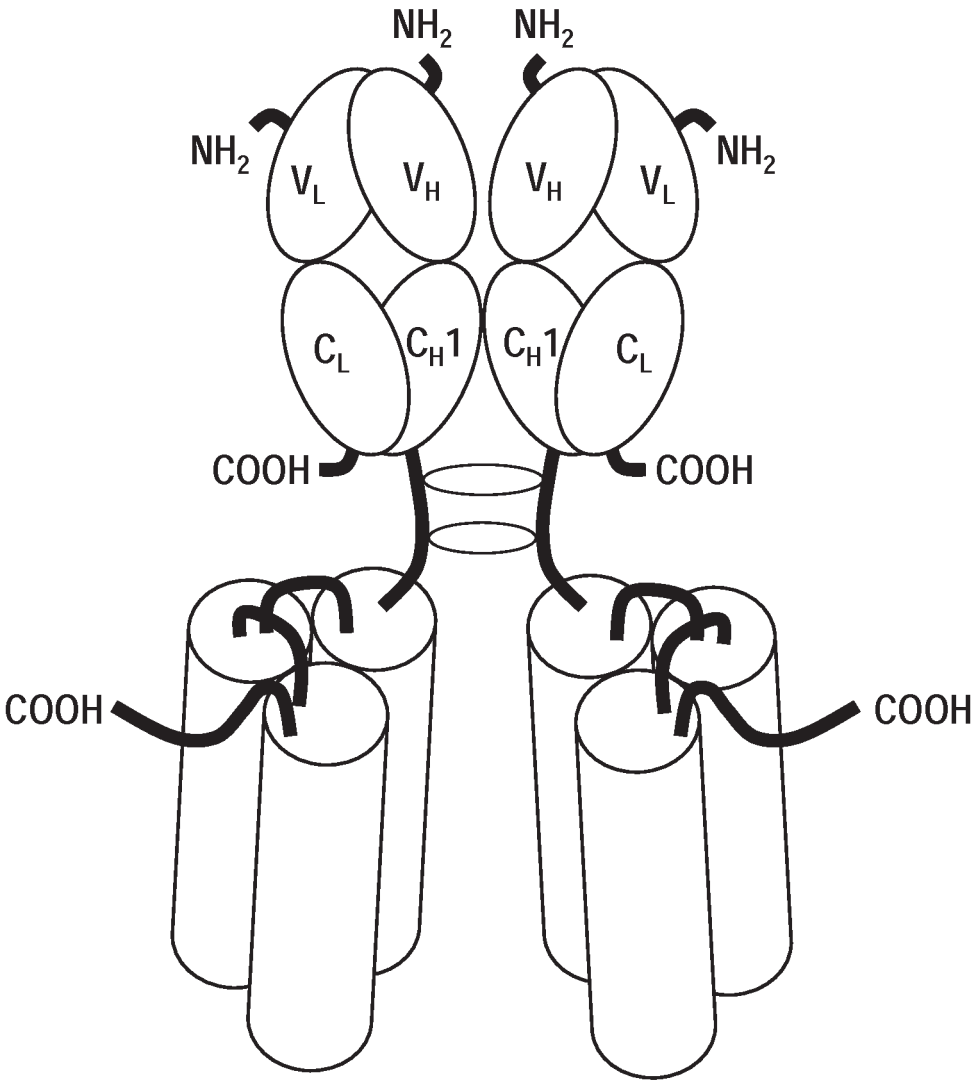


Fig. 15

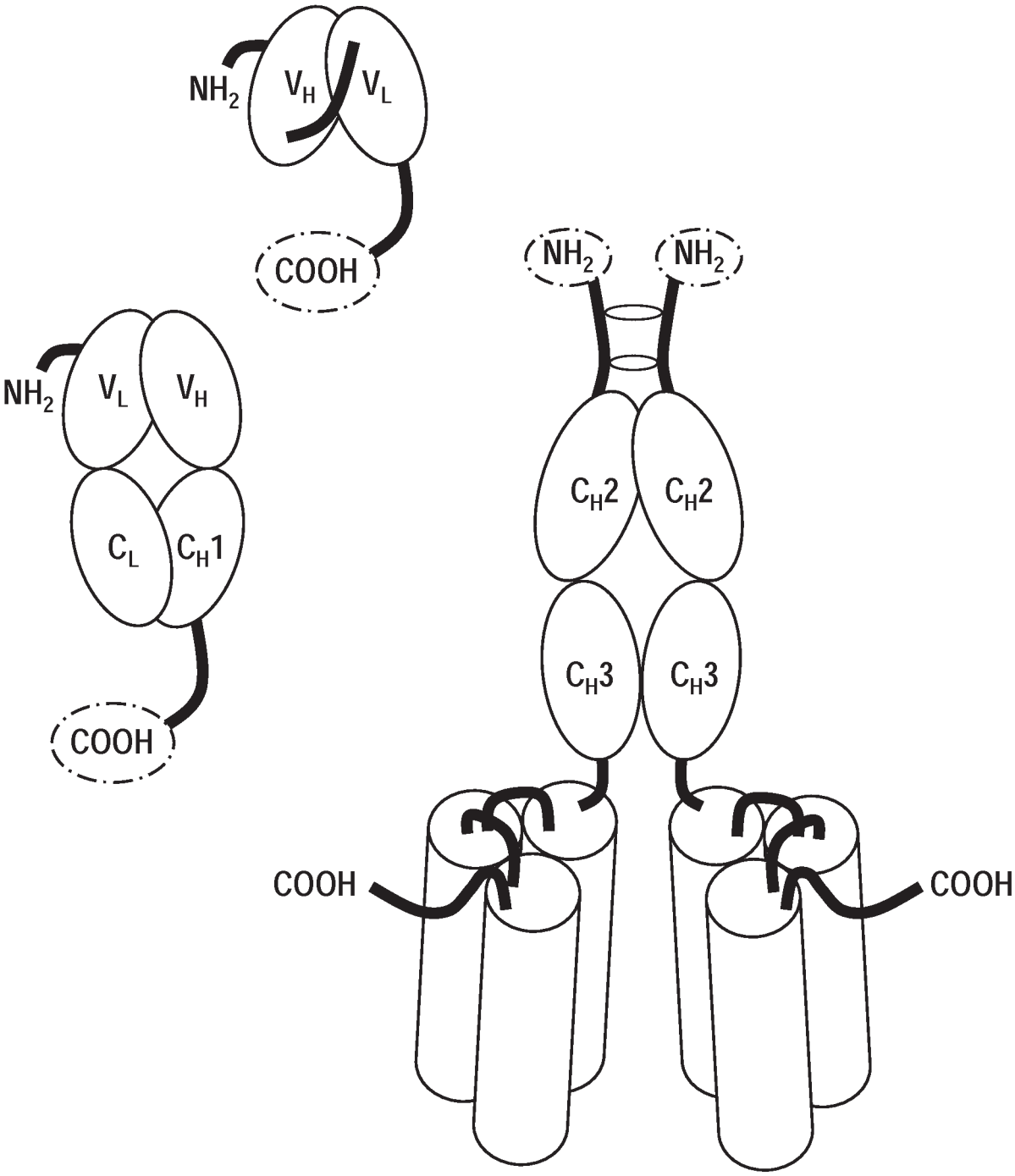
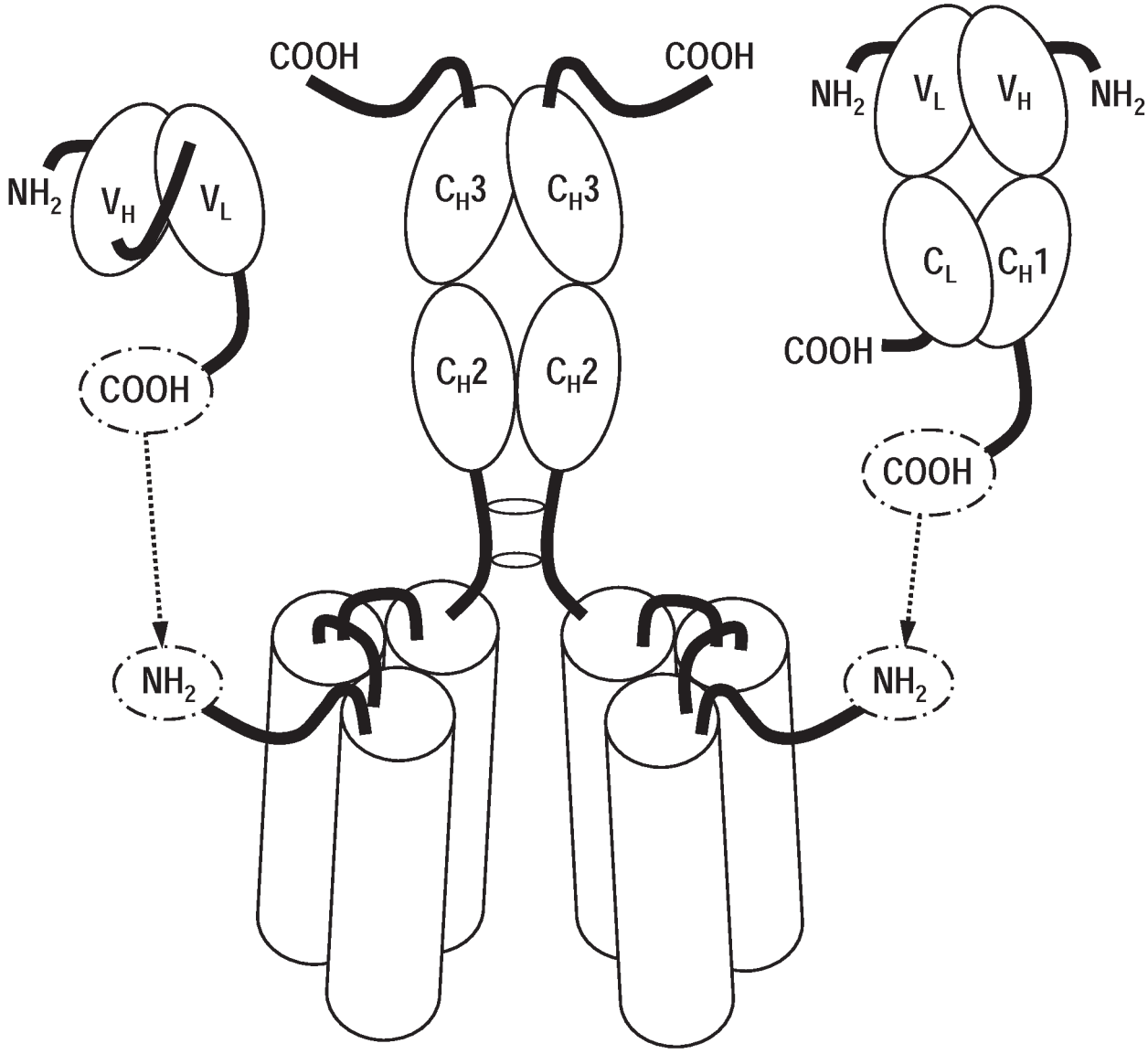

NH2
VH
VL
COOH
NH2
VL
VH
CL
CH1
COOH
NH2
NH2
CH2
CH2
CH3
CH3
COOH
COOH

Fig. 16


COOH
COOH
NH2
VH
VL
CH3
CH3
NH2
VL
VH
NH2
CL
CH1
CH2
CH2
COOH
COOH
COOH
NH2
NH2

Signal peptide is displayed in **bolded and underlined text** (not present in the mature protein): **SEQ ID NO: 12**
TRAIL receptor binding domain monomer modules in plain text: as 120-281 and 121-281 of SEQ ID NO:1, respectively
linkers between the TRAIL monomers in bolded text: **SEQ ID NO:2**
Fc-domain of human IgG1 in *italicized and underlined text: SEQ ID NO: 10*
Hinge-linker between the scTRAIL –RBD and Fc is displayed in ***bolded and ilalicized text: SEQ ID NO: 11***

METDTLLVFVLLVWVPAGNGQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGH
SFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLM
KSARNSCWSKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG**GSGS**
GNGSRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHE
KGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY
SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG**GSGSGNGS**RVAAHITGTRGRS
NTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQEE
IKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKENDRIF
VSVTNEHLIDMDHEASFFGAFLVG***GPGSSSSSSSSGSCDKTHTCPPC****PAPELLGGPSVFLFPP*
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYSSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
*LHNHYTQKSLSLSPGK**

Fig. 17A

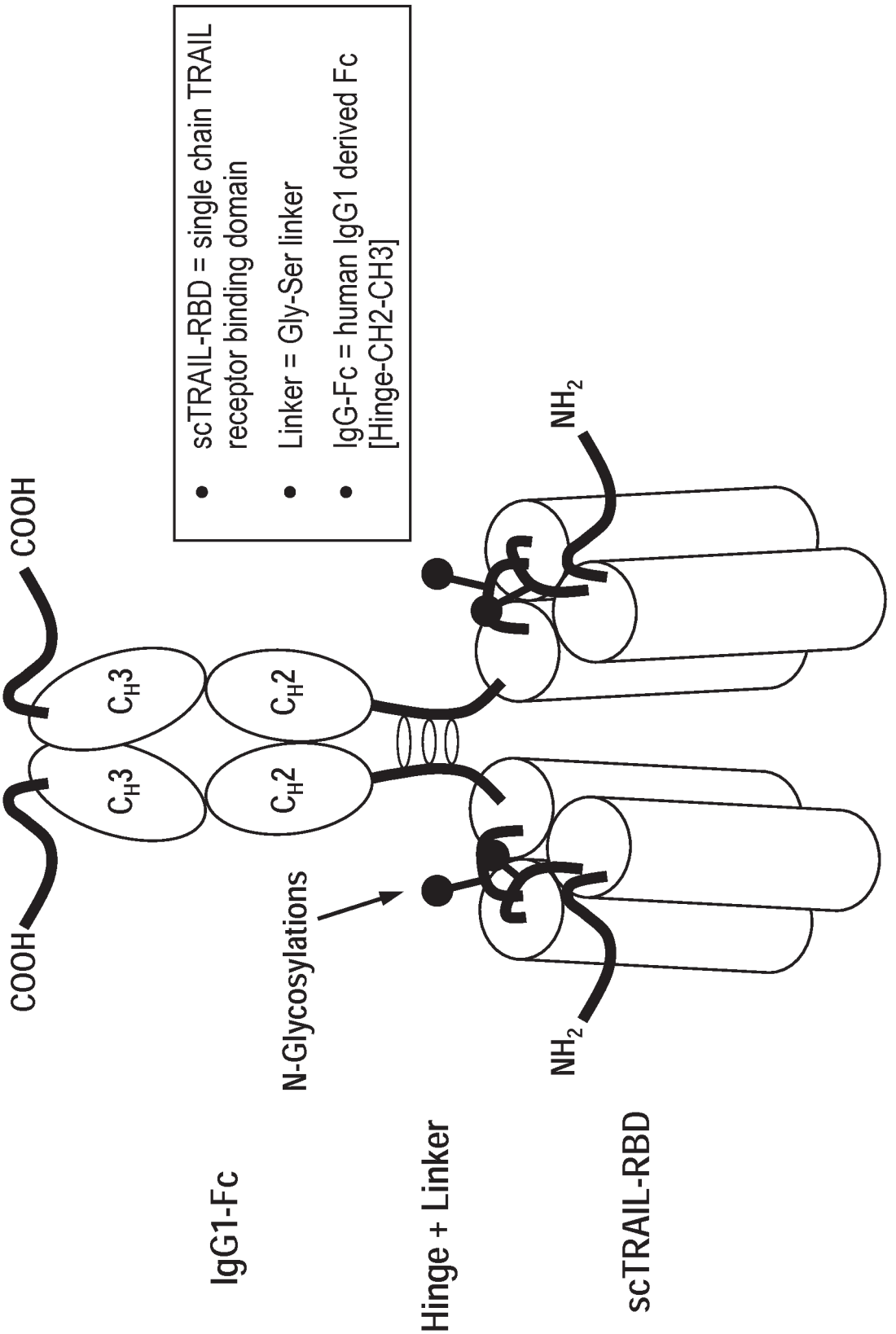

• scTRAIL-RBD = single chain TRAIL receptor binding domain
• Linker = Gly-Ser linker
• IgG-Fc = human IgG1 derived Fc [Hinge-CH2-CH3]
COOH
COOH
C_H3
C_H3
C_H2
C_H2
N-Glycosylations
NH_2
NH_2
IgG1-Fc
Hinge + Linker
scTRAIL-RBD

Fig. 17B

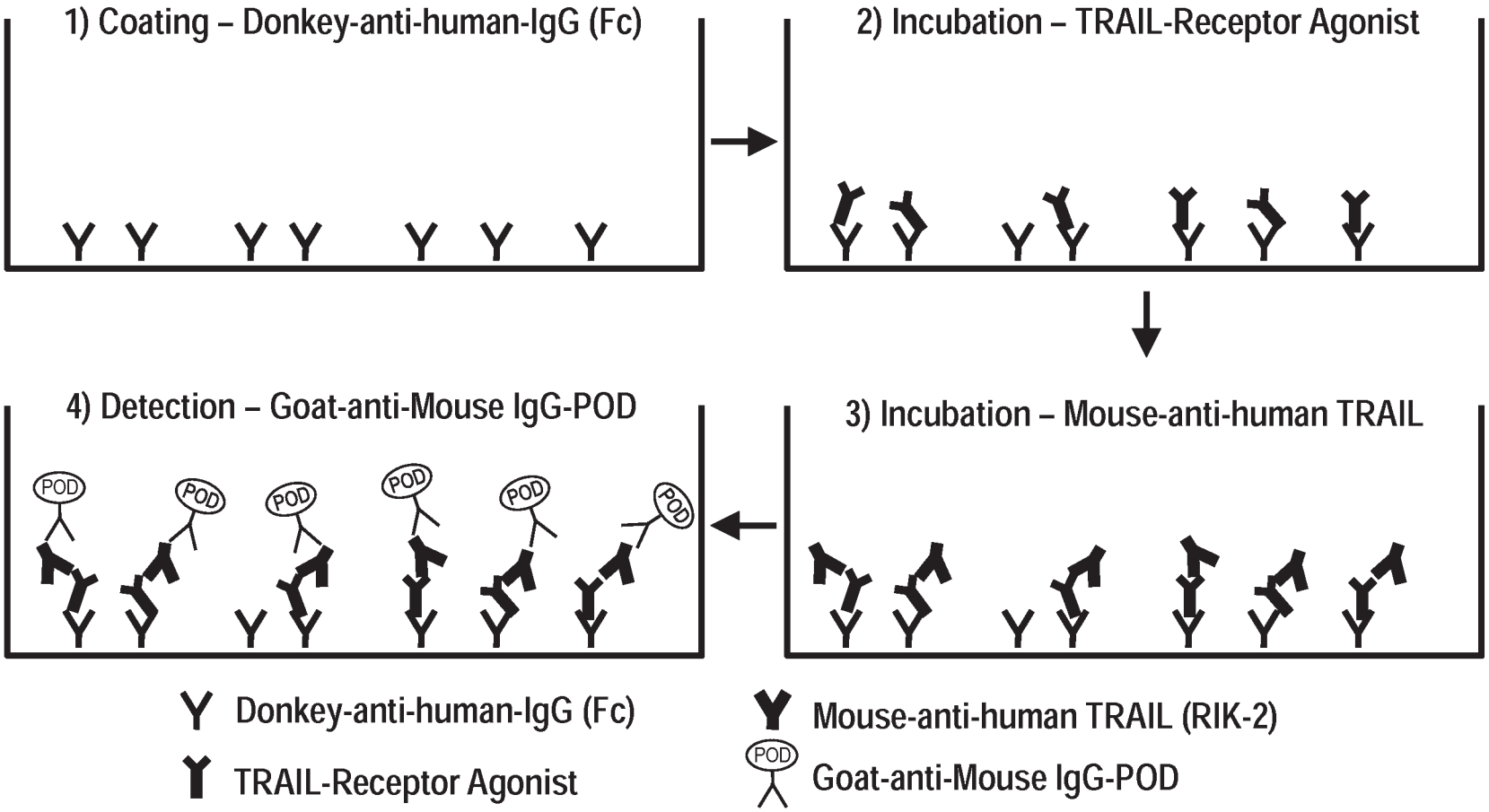
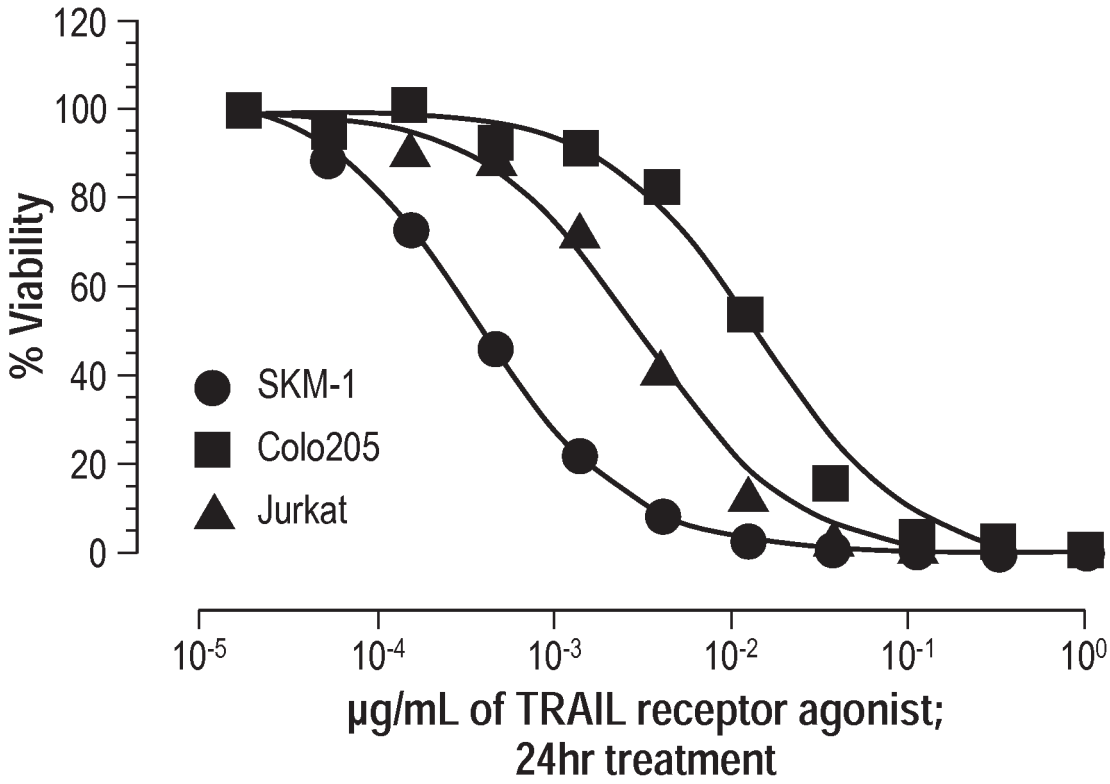

1) Coating – Donkey-anti-human-IgG (Fc)
2) Incubation – TRAIL-Receptor Agonist
3) Incubation – Mouse-anti-human TRAIL
4) Detection – Goat-anti-Mouse IgG-POD
POD
Donkey-anti-human-IgG (Fc)
TRAIL-Receptor Agonist
Mouse-anti-human TRAIL (RIK-2)
Goat-anti-Mouse IgG-POD

Fig. 18

Fig. 19


120
100
80
60
40
20
0
% Viability
SKM-1
Colo205
Jurkat
10-5
10-4
10-3
10-2
10-1
100
µg/mL of TRAIL receptor agonist;
24hr treatment

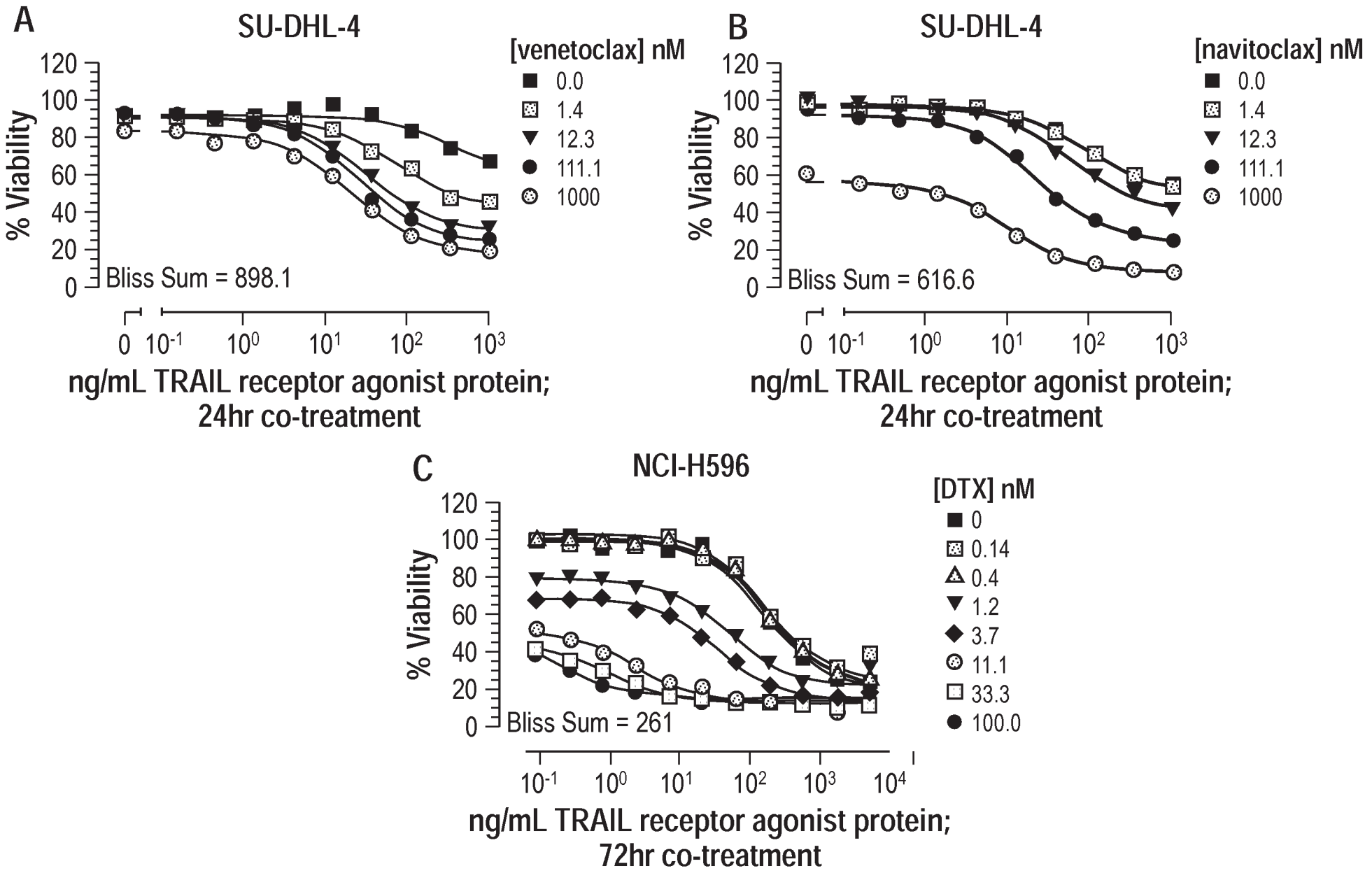

A
SU-DHL-4
[venetoclax] nM
0.0
1.4
12.3
111.1
1000
% Viability
Bliss Sum = 898.1
ng/mL TRAIL receptor agonist protein;
24hr co-treatment
B
SU-DHL-4
[navitoclax] nM
0.0
1.4
12.3
111.1
1000
% Viability
Bliss Sum = 616.6
ng/mL TRAIL receptor agonist protein;
24hr co-treatment
C
NCI-H596
[DTX] nM
0
0.14
0.4
1.2
3.7
11.1
33.3
100.0
% Viability
Bliss Sum = 261
ng/mL TRAIL receptor agonist protein;
72hr co-treatment

Fig. 20

Fig. 21

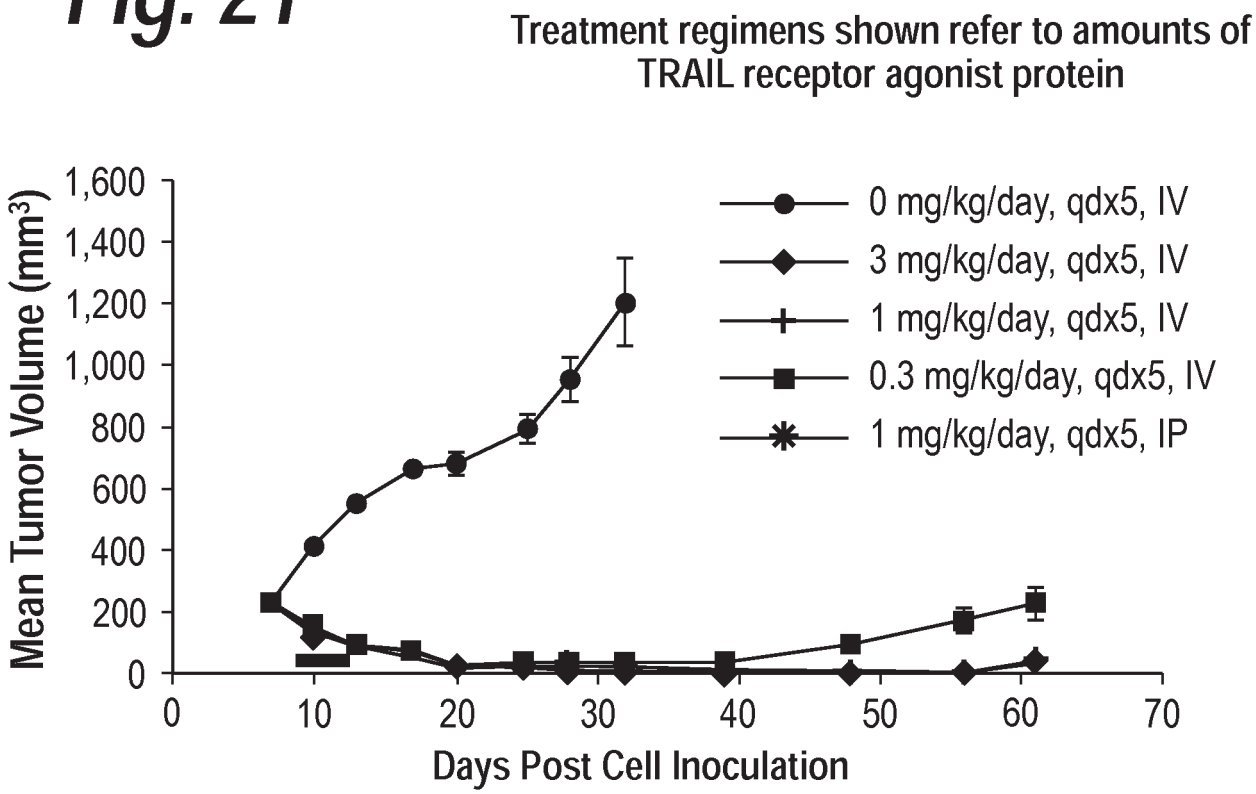

Treatment regimens shown refer to amounts of TRAIL receptor agonist protein
Mean Tumor Volume (mm3)
1,600
1,400
1,200
1,000
800
600
400
200
0
0
10
20
30
40
50
60
70
Days Post Cell Inoculation
0 mg/kg/day, qdx5, IV
3 mg/kg/day, qdx5, IV
1 mg/kg/day, qdx5, IV
0.3 mg/kg/day, qdx5, IV
1 mg/kg/day, qdx5, IP

Fig. 22

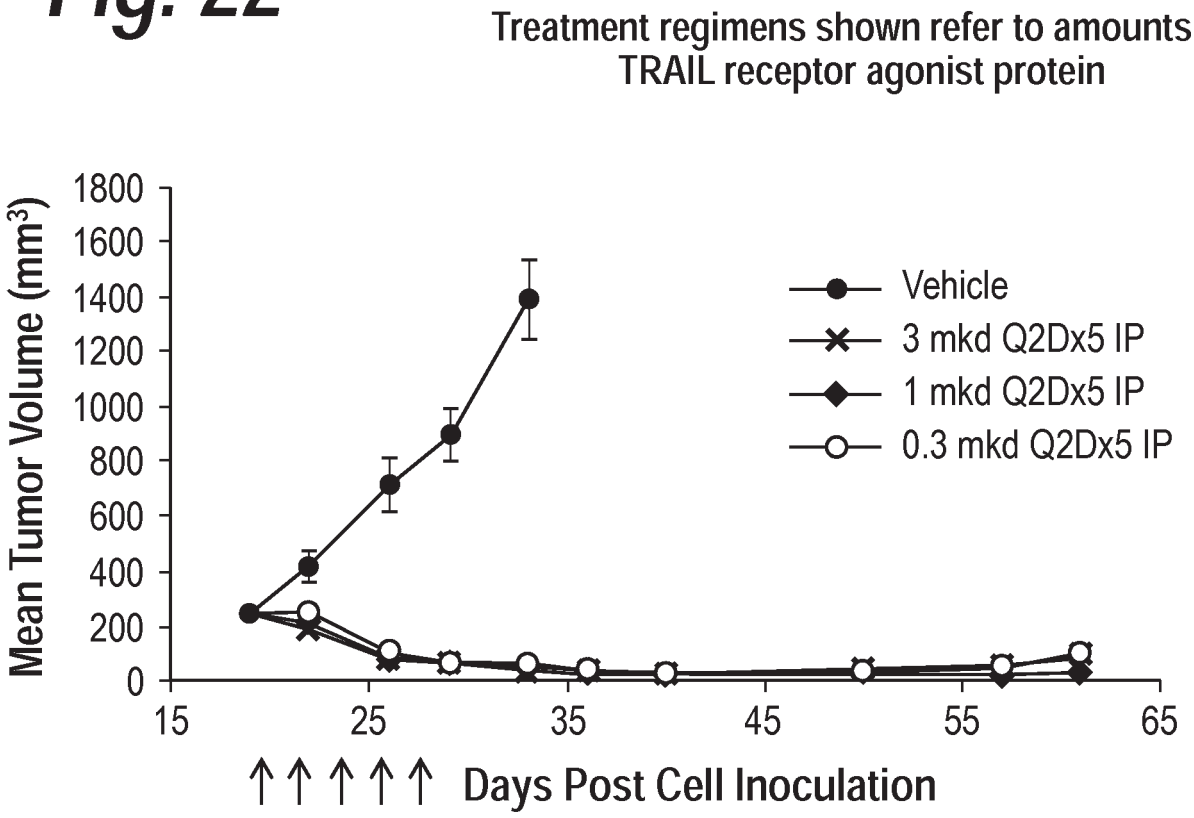
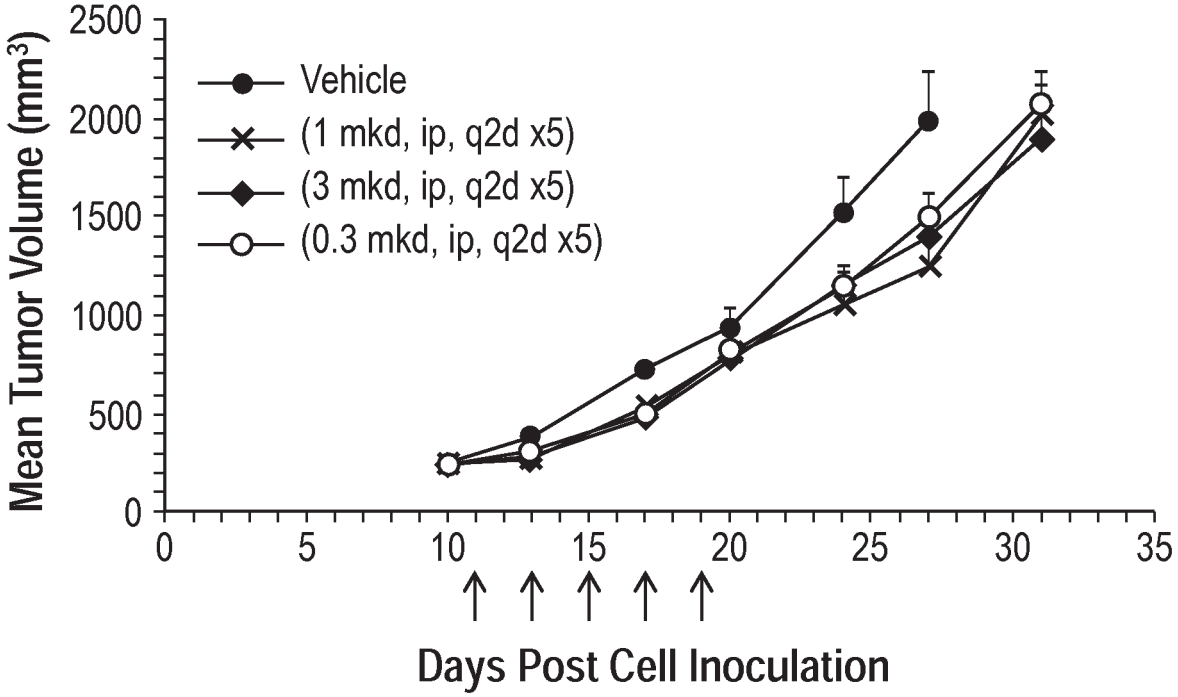

Treatment regimens shown refer to amounts of TRAIL receptor agonist protein
Mean Tumor Volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
15
25
35
45
55
65
Days Post Cell Inoculation
Vehicle
3 mkd Q2Dx5 IP
1 mkd Q2Dx5 IP
0.3 mkd Q2Dx5 IP

Fig. 23

Treatment regimens shown refer to amounts of TRAIL receptor agonist protein



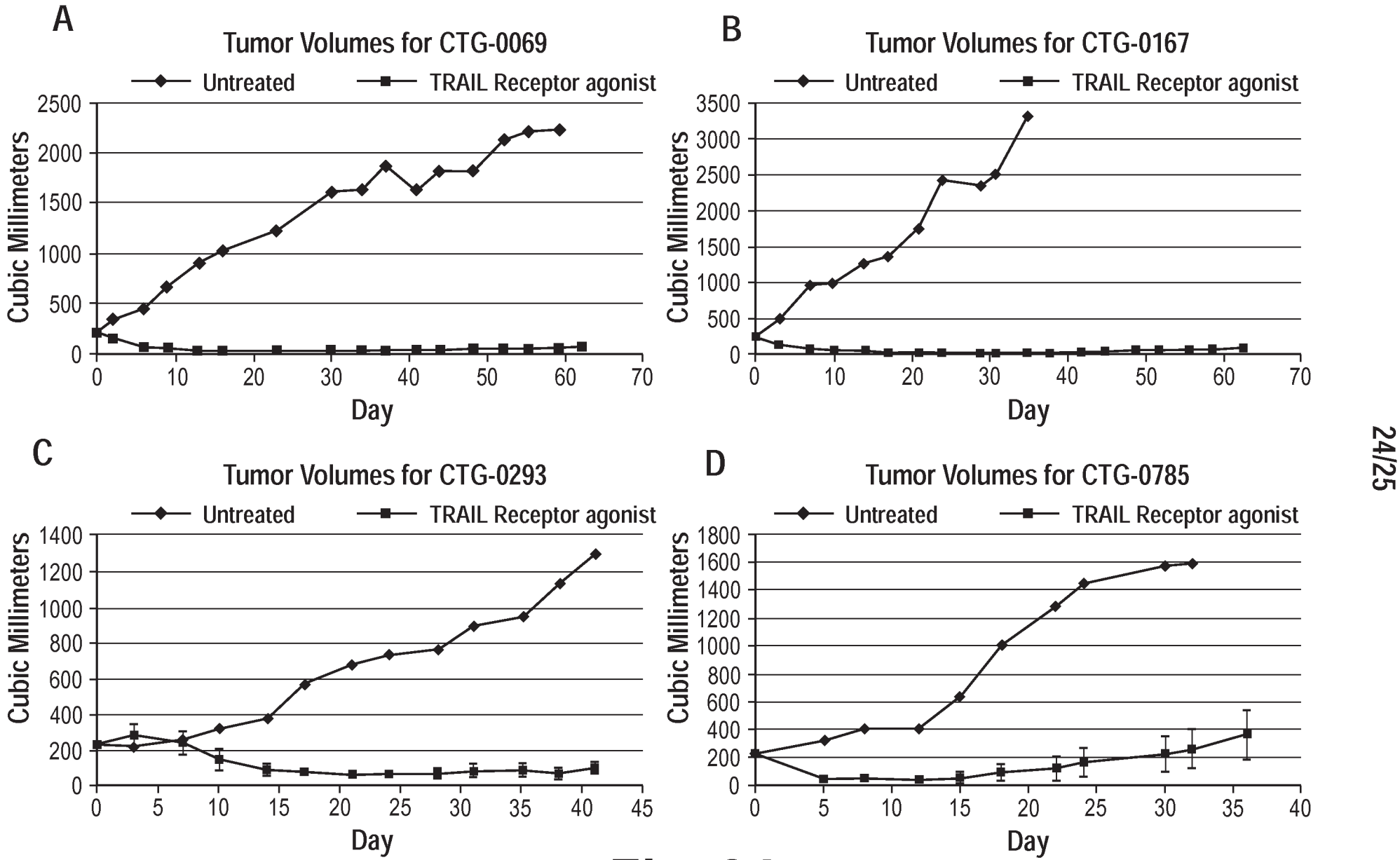

A
Tumor Volumes for CTG-0069
Untreated
TRAIL Receptor agonist
Cubic Millimeters
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
Day
B
Tumor Volumes for CTG-0167
Untreated
TRAIL Receptor agonist
Cubic Millimeters
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
Day
C
Tumor Volumes for CTG-0293
Untreated
TRAIL Receptor agonist
Cubic Millimeters
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Day
D
Tumor Volumes for CTG-0785
Untreated
TRAIL Receptor agonist
Cubic Millimeters
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
Day

Fig. 24

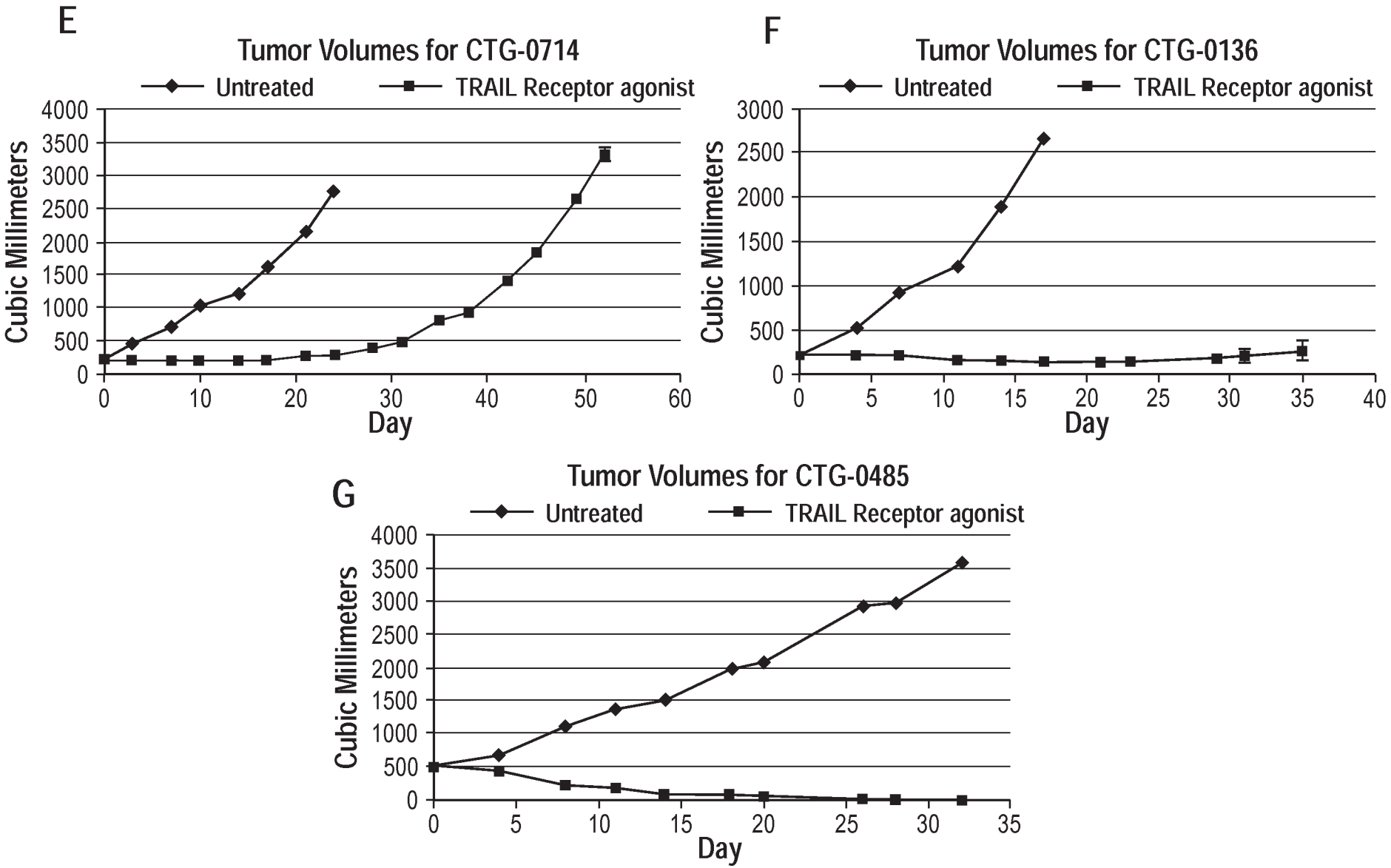

E
Tumor Volumes for CTG-0714
Untreated
TRAIL Receptor agonist
Cubic Millimeters
Day
F
Tumor Volumes for CTG-0136
Untreated
TRAIL Receptor agonist
Cubic Millimeters
Day
G
Tumor Volumes for CTG-0485
Untreated
TRAIL Receptor agonist
Cubic Millimeters
Day

Fig. 24