[2023-1학기 파란학기제]

AI-based Program을 활용한 IL-2 Protein Engineering

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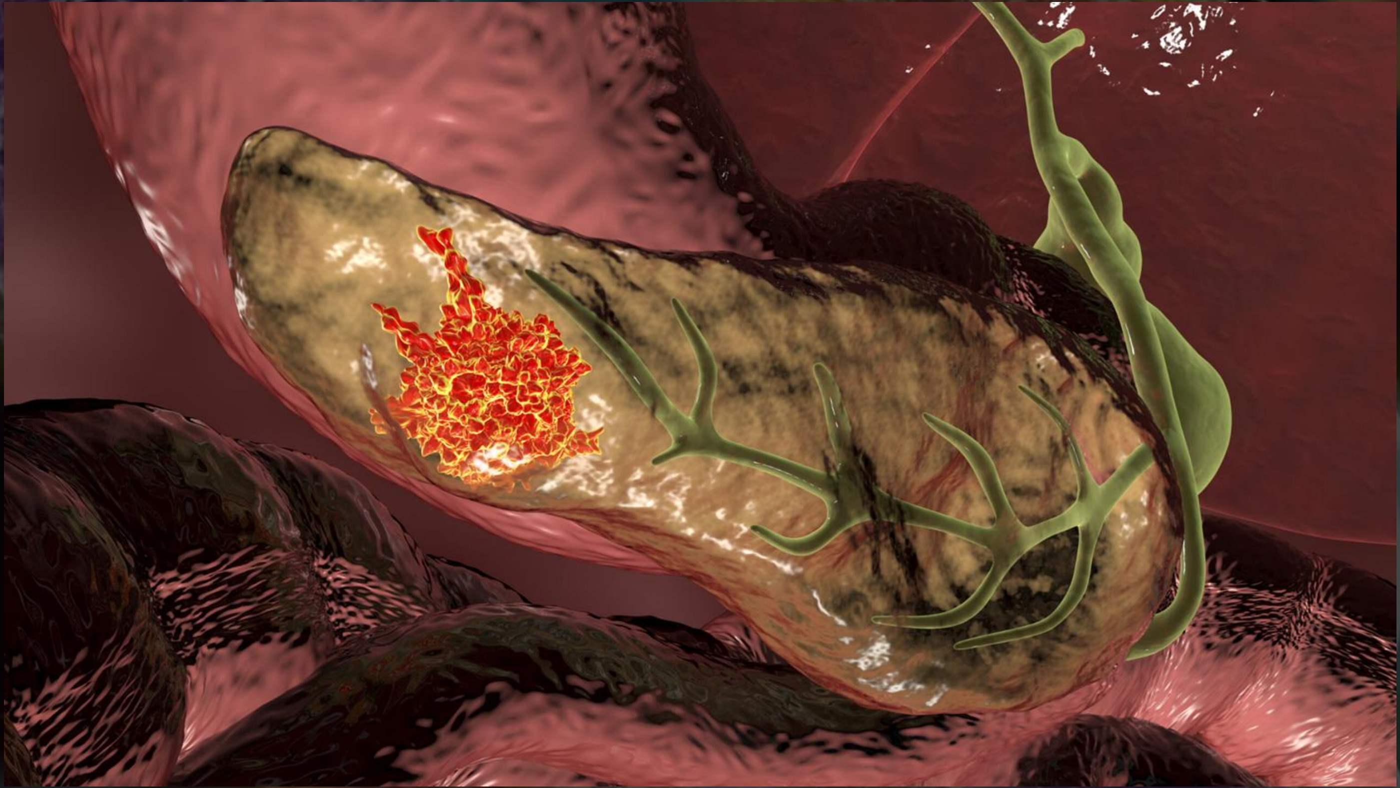
생명과학과 3학년 정현정

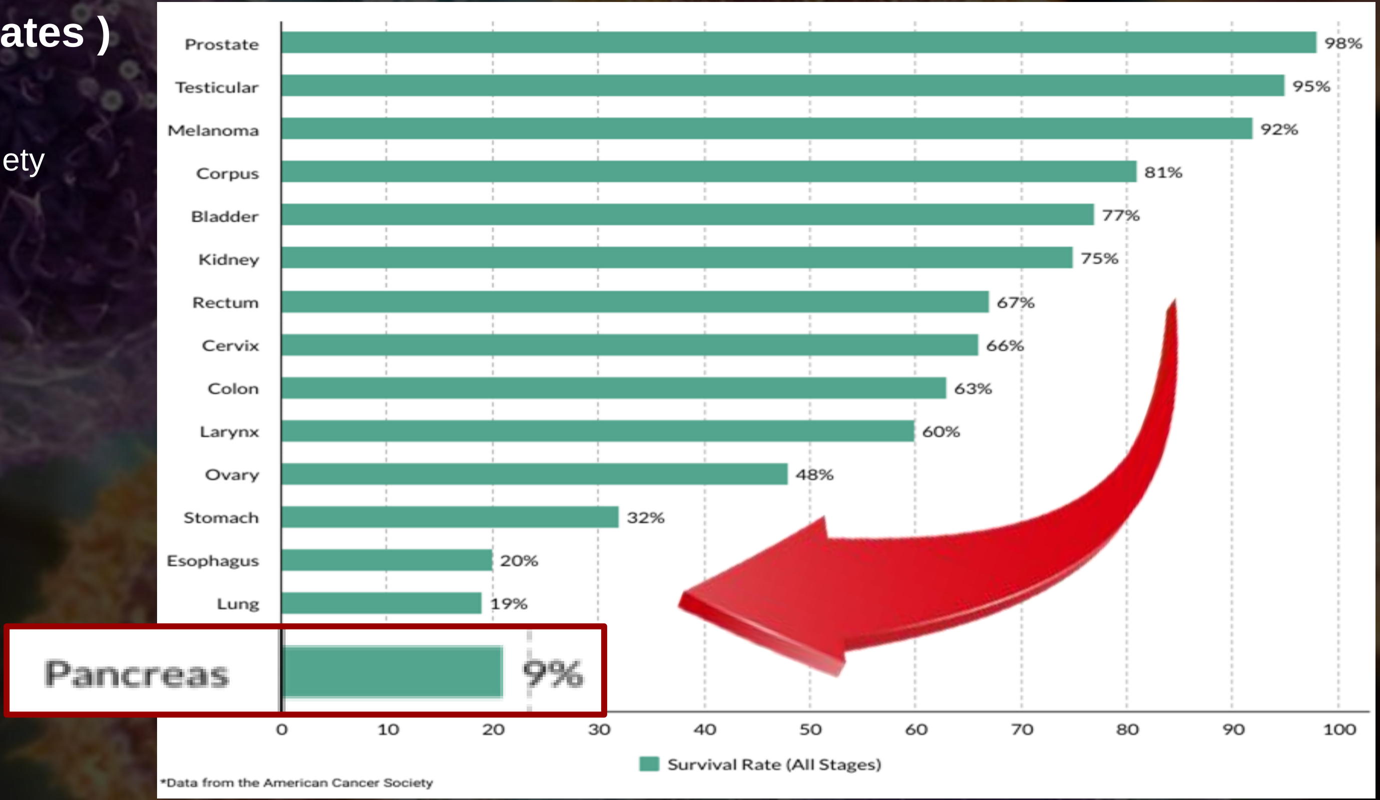
Pancreatic Cancer Immunotherapy



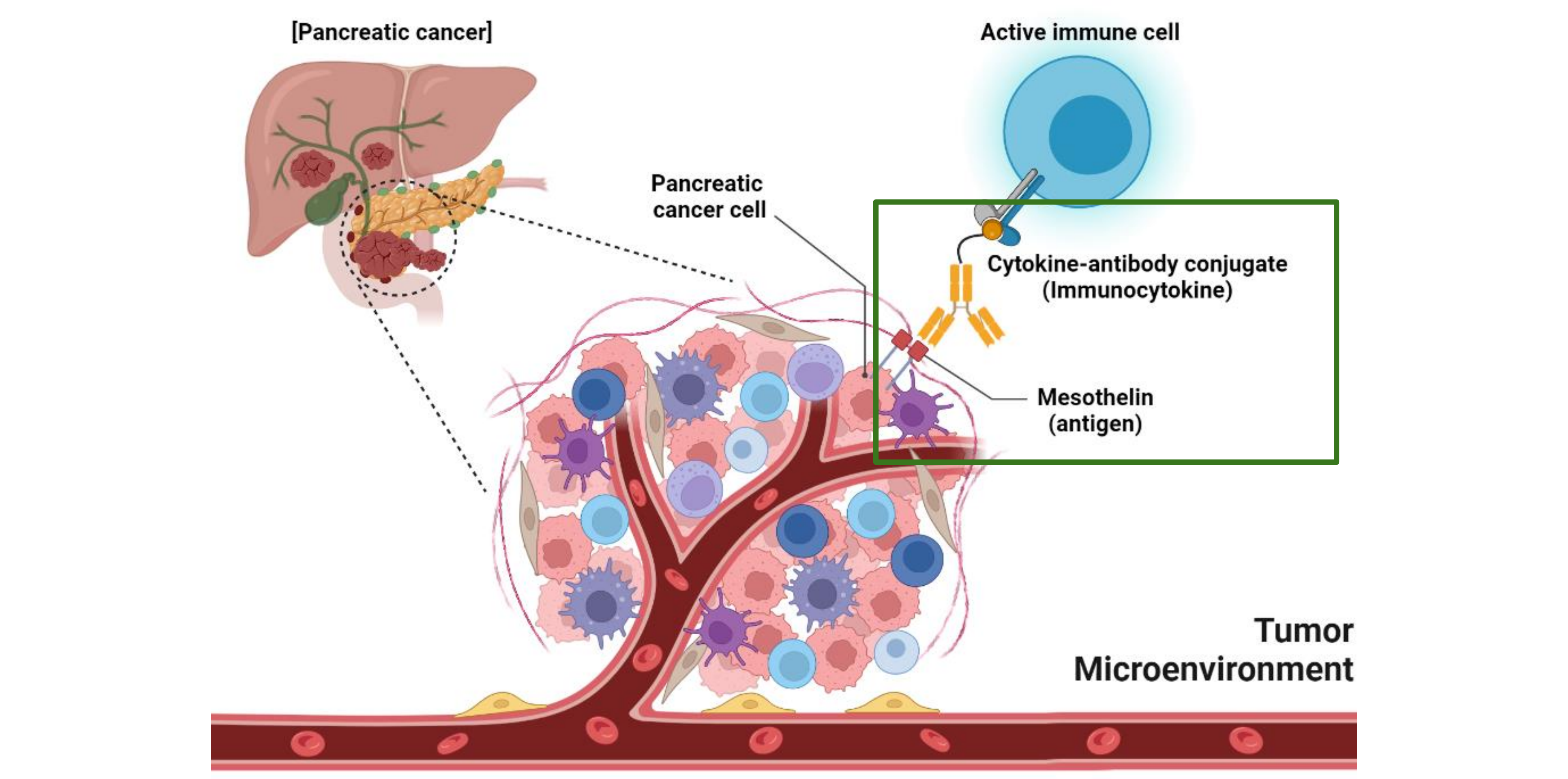
Pancreatic Cancer Immunotherapy

5-year Survival Rates)

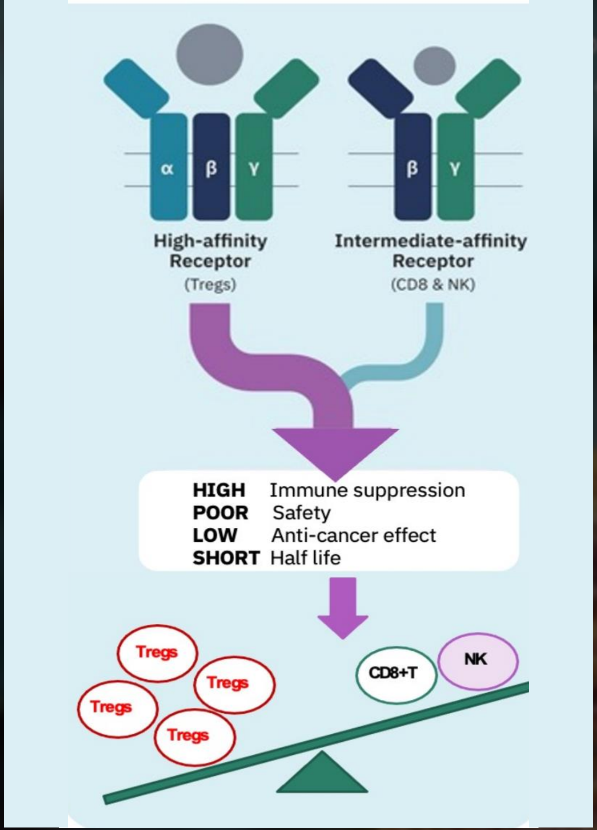
* Data from the
  American Cancer Society

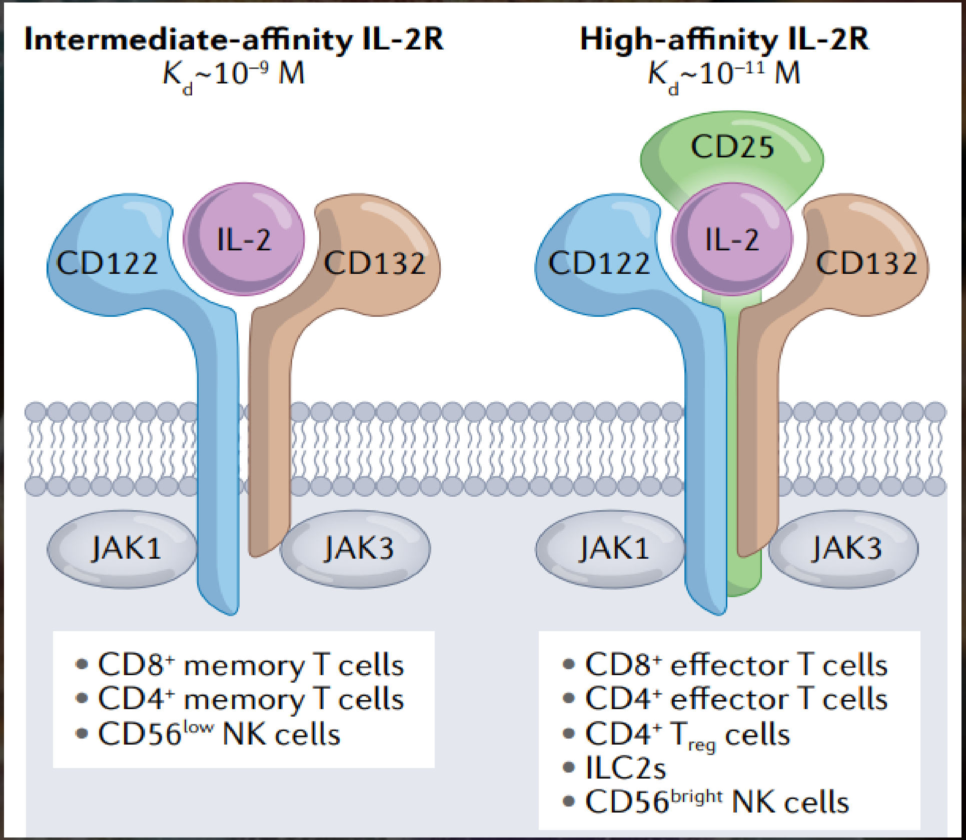


Pancreatic Cancer Immunocytokine



IL-2 pleiotropy

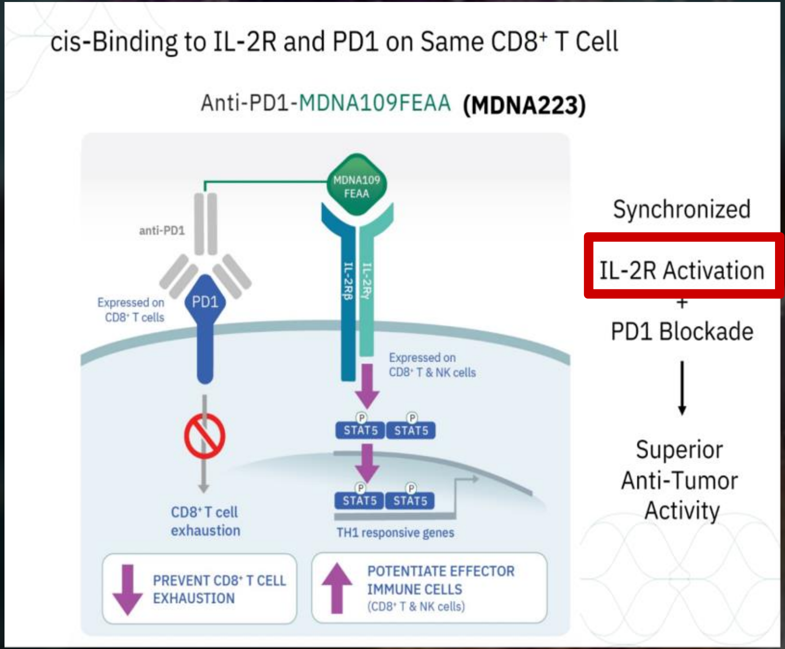


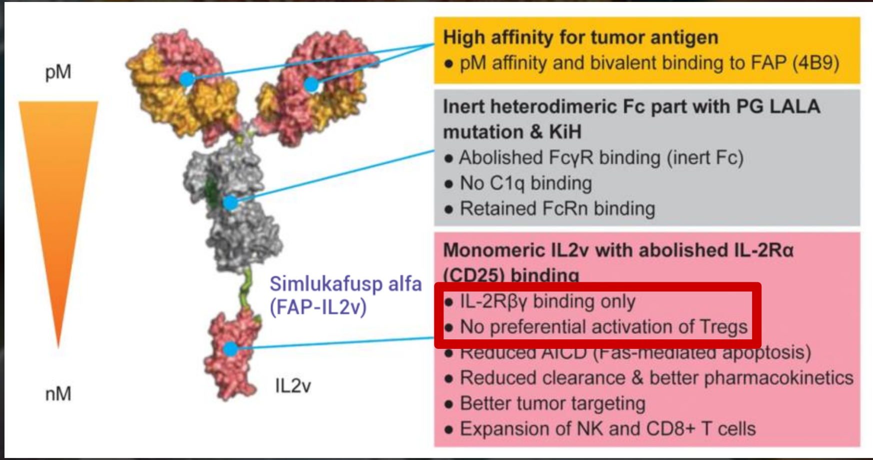


Global Big-pharma

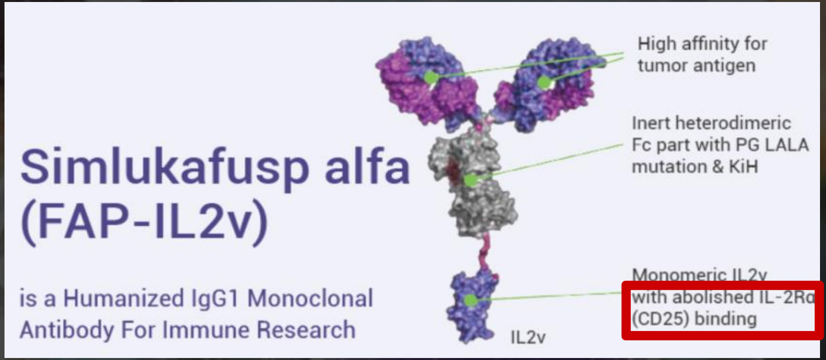
[Roche]

[MEDICENNA]

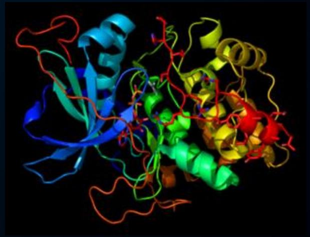


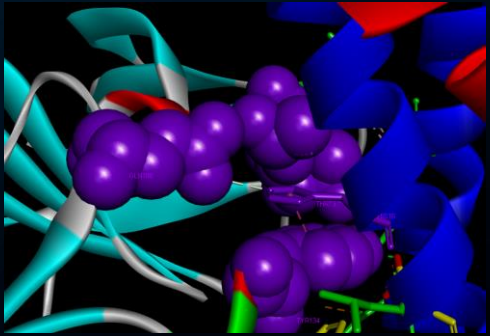


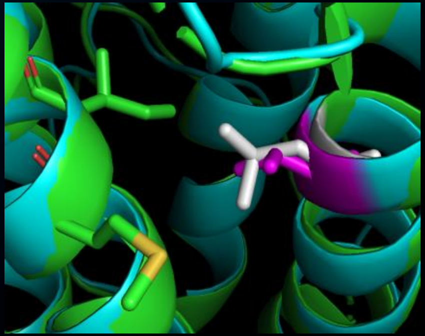
[Roche]



AI & Computational Design in In-silico

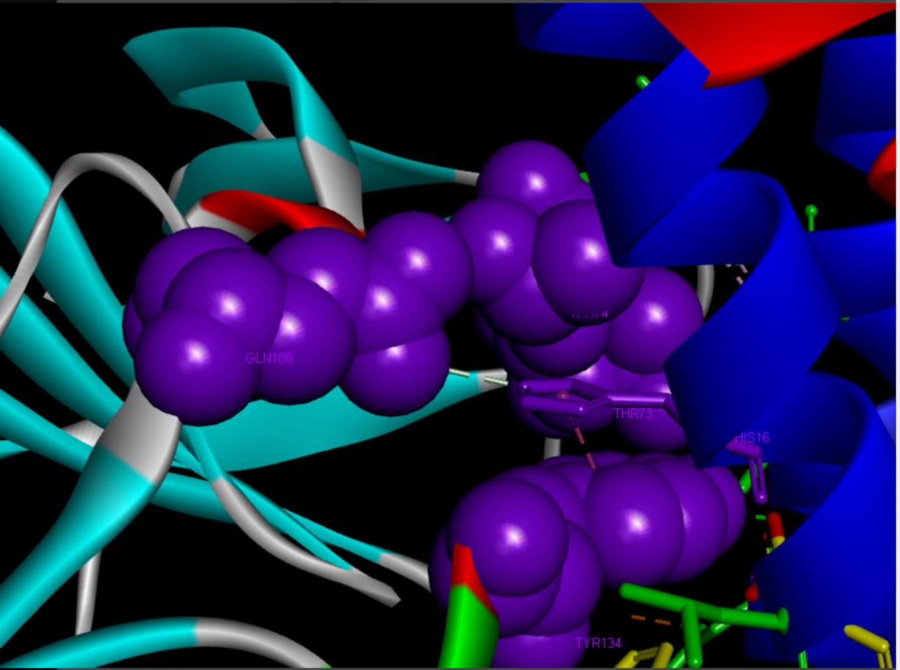


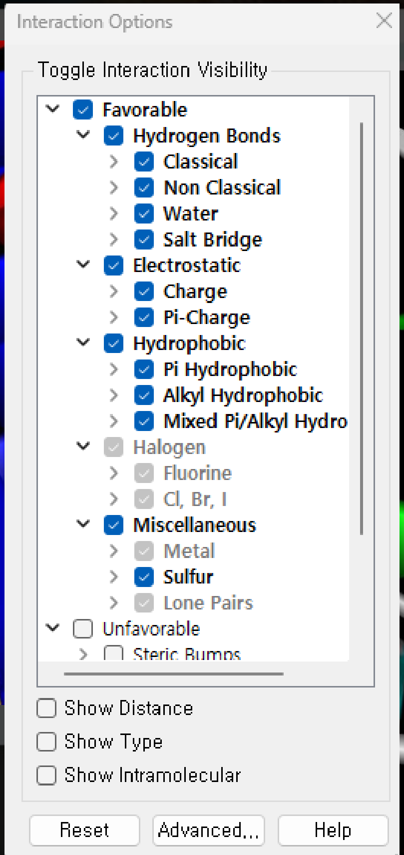


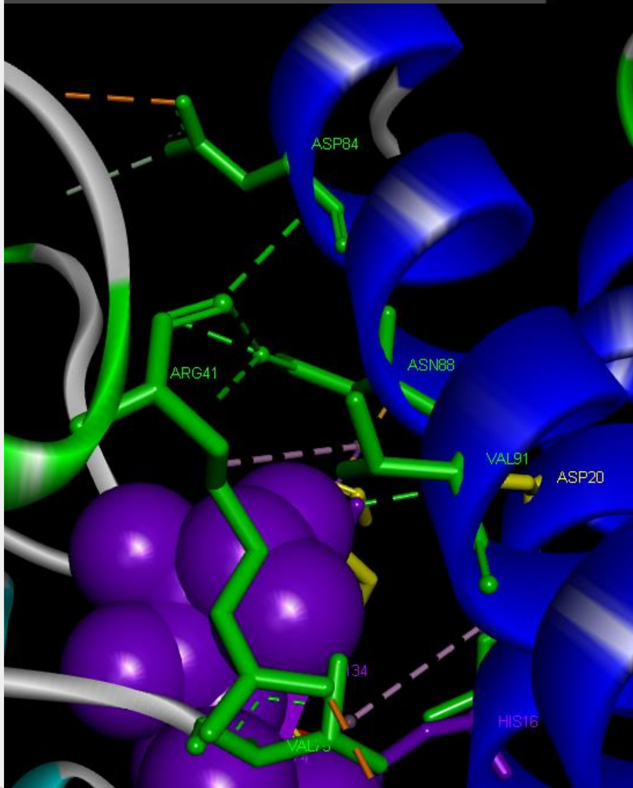


Visualization - Discovery Studio

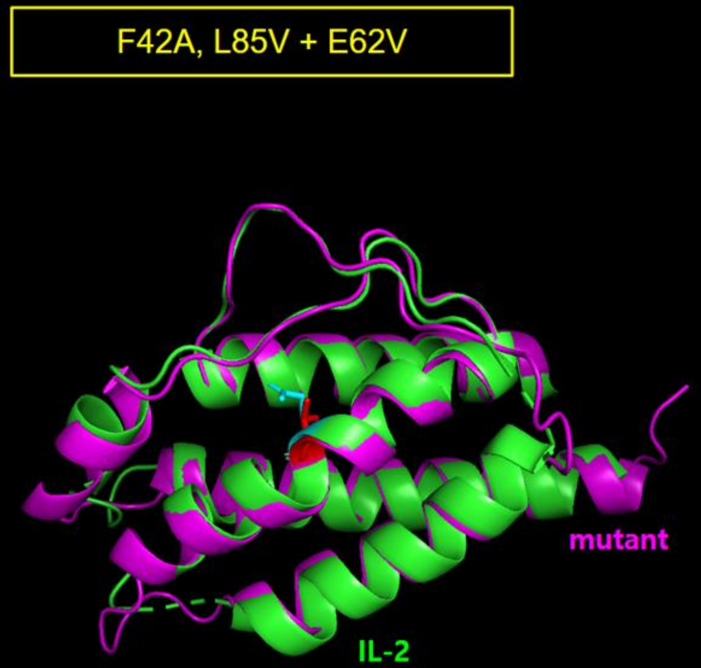
IL-2Rb - IL-2 interaction

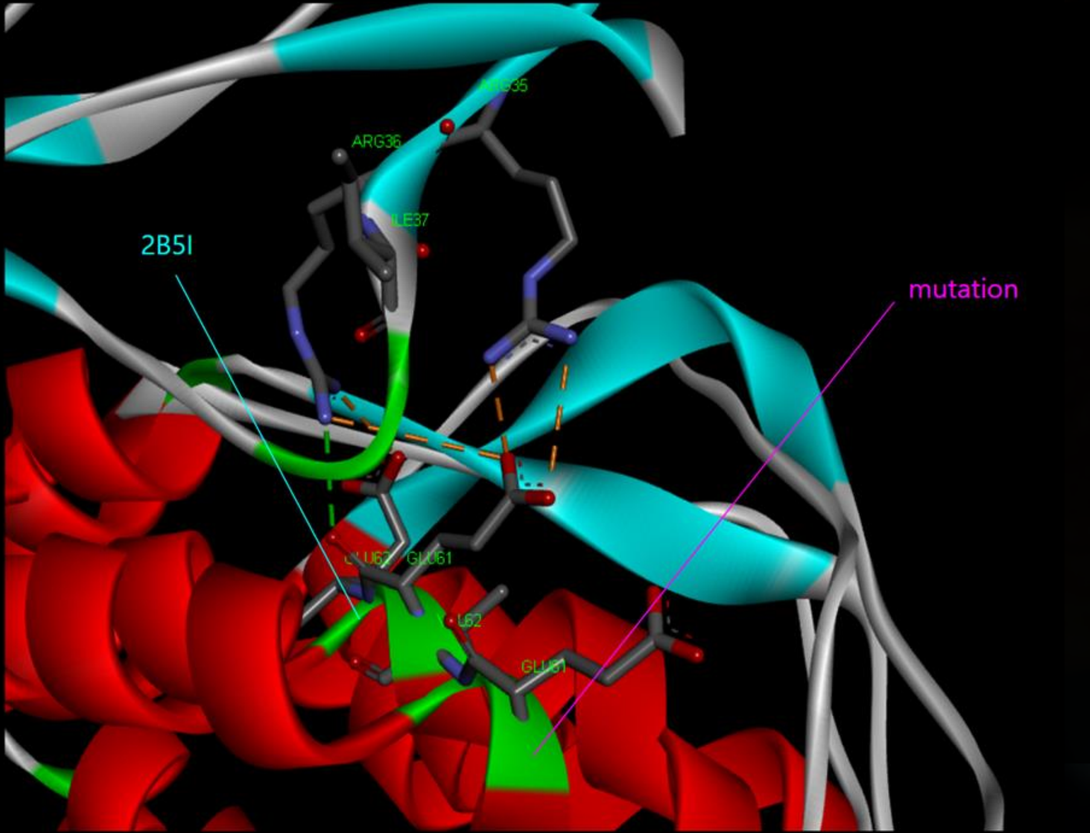




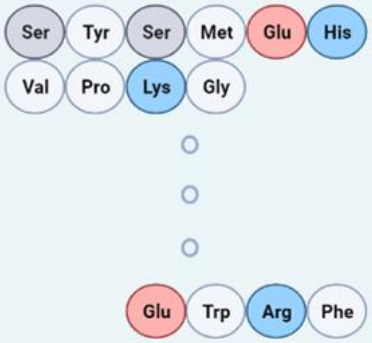


AlphaFold2 / PyMOL / Discovery Studio

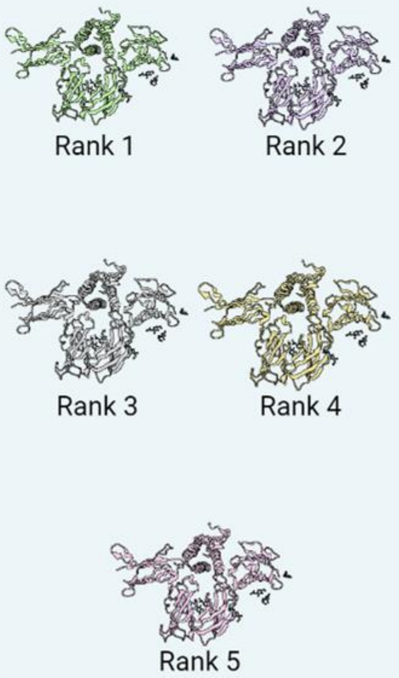




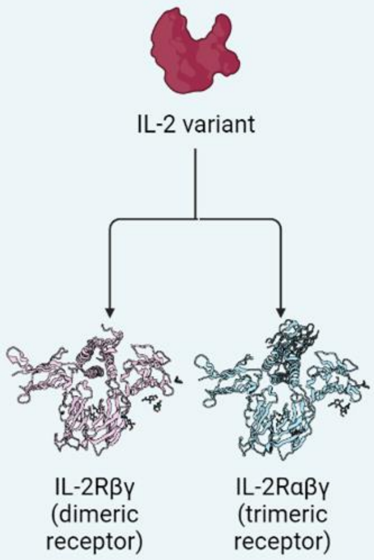
1] variant 서열 생성



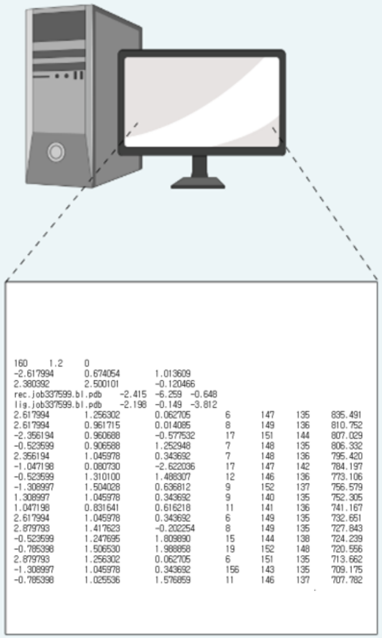
2] variant 구조 예측



3] docking



4] 결합 점수 산출



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-2.617994	0.674054	1.013609				
2.380392	2.500101	-0.120466				
rec.job337599.bl.pdb	-2.415	-6.259	-0.648			
lig.job337599.bl.pdb	-2.198	-0.149	-3.812			
2.617994	1.256302	0.062705	6	147	135	835.491
2.617994	0.961715	0.014085	8	149	136	810.752
-2.356194	0.960688	-0.577532	17	151	144	807.029
-0.523599	0.906588	1.252948	7	148	135	806.332
2.356194	1.045978	0.343692	7	148	136	795.420
-1.047198	0.080730	-2.622036	17	147	142	784.197
-0.523599	1.310100	1.488307	12	146	136	773.106
-1.308997	1.504028	0.636812	9	152	137	756.579
1.308997	1.045978	0.343692	9	140	135	752.305
1.047198	0.831641	0.616218	11	141	136	741.167
2.617994	1.045978	0.343692	6	149	135	732.651
2.879793	1.417623	-0.202254	8	149	135	727.843
-0.523599	1.247695	1.809890	15	144	138	724.239
-0.785398	1.506530	1.988858	19	152	148	720.556
2.879793	1.256302	0.062705	6	151	135	713.662
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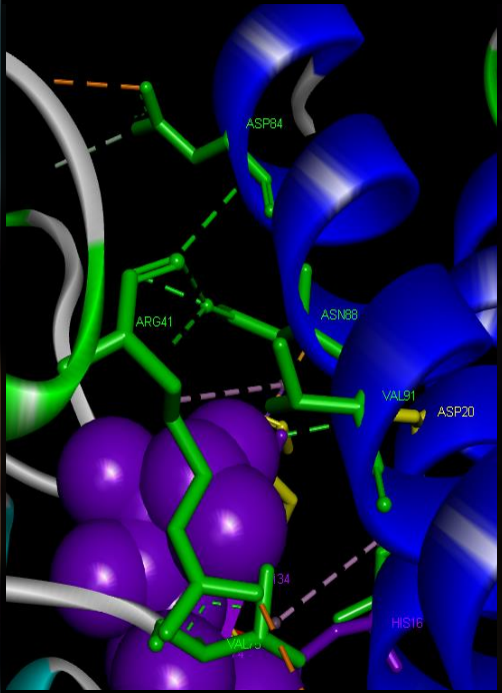
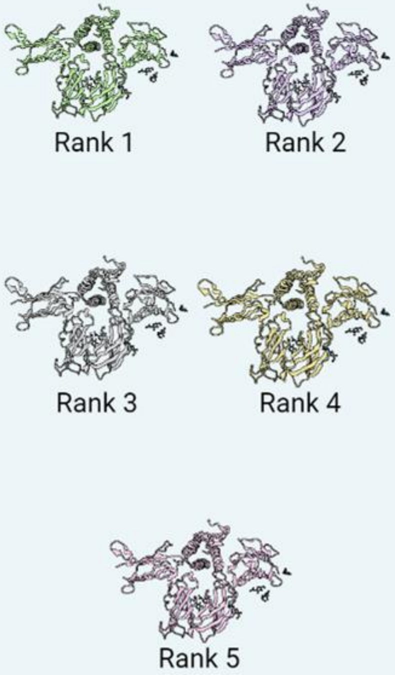
-0.785398	1.025536	1.576859	11	146	137	707.782

Discovery Studio



2] variant 구조 예측



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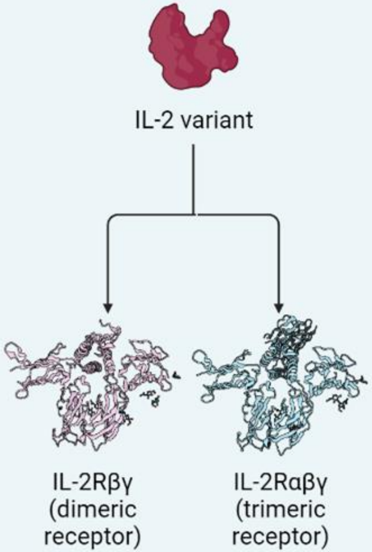

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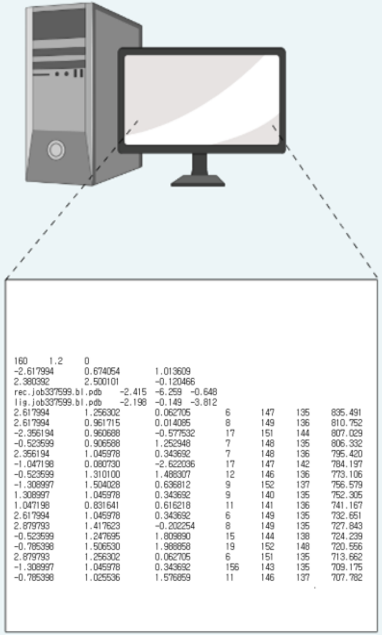
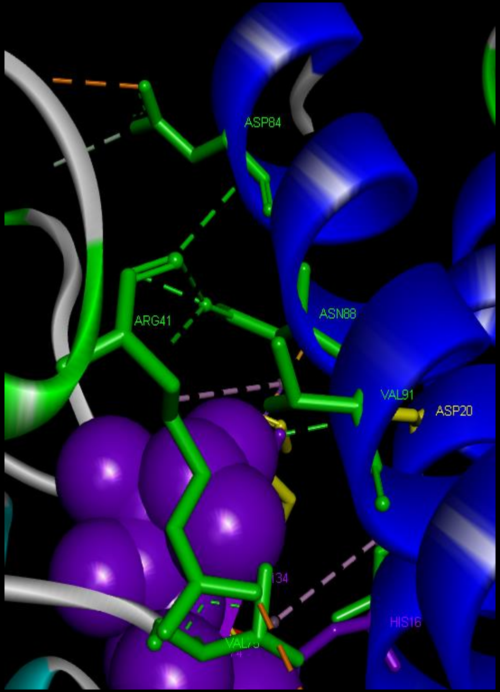
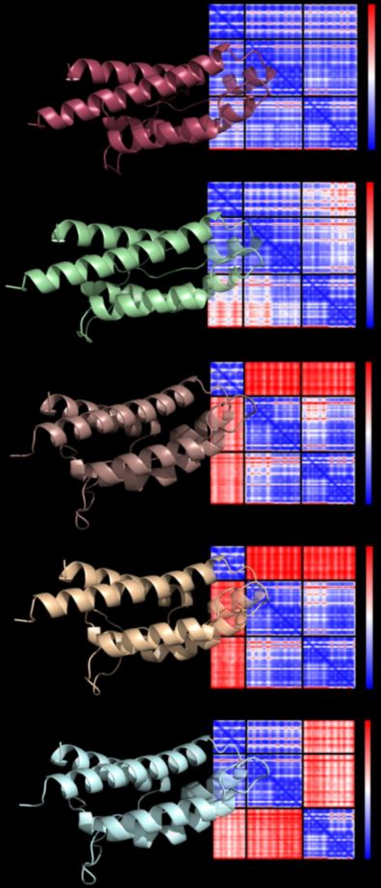


Discovery Studio



ColabFold.adv



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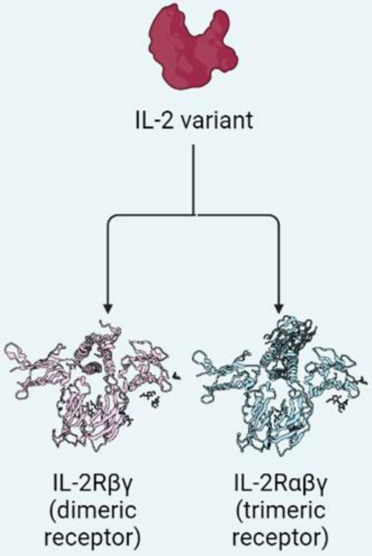

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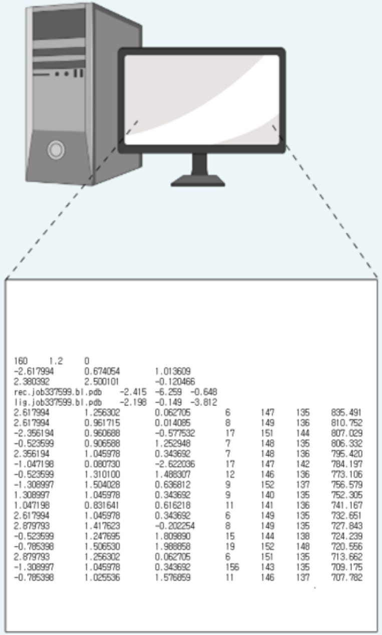

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-2.617994      0.674054      1.013609
2.380392       2.500101      -0.120466
rec.job337599.bl.pdb    -2.415  -6.259  -0.648
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2.617994       1.256302      0.062705      6      147     135     835.491
2.617994       0.961715      0.014085      8      149     136     810.752
-2.356194      0.960688      -0.577532     17     151     144     807.029
-0.523599      0.906588      1.252948      7      148     135     806.332
2.356194       1.045978      0.343692      7      148     136     795.420
-1.047198      0.080730      -2.622036     17     147     142     784.197
-0.523599      1.310100      1.488307      12     146     136     773.106
-1.308997      1.504028      0.636812      9      152     137     756.579
1.308997       1.045978      0.343692      9      140     135     752.305
1.047198       0.831641      0.616218      11     141     136     741.167
2.617994       1.045978      0.343692      6      149     135     732.651
2.879793       1.417623      -0.202254     8      149     135     727.843
-0.523599      1.247695      1.809890      15     144     138     724.239
-0.785398      1.506530      1.988858      19     152     148     720.556
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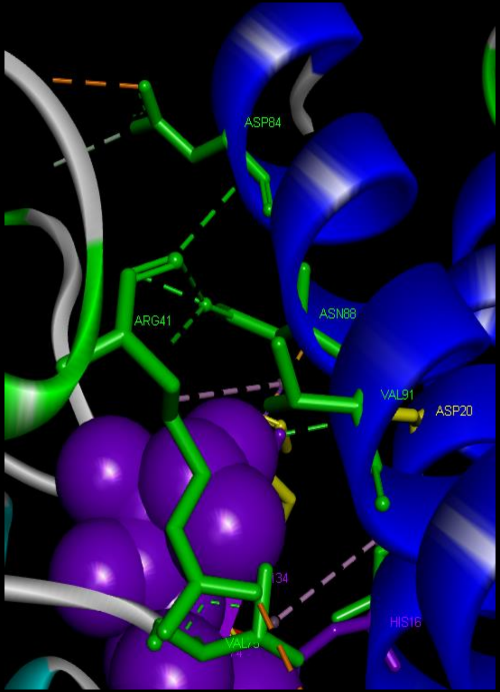
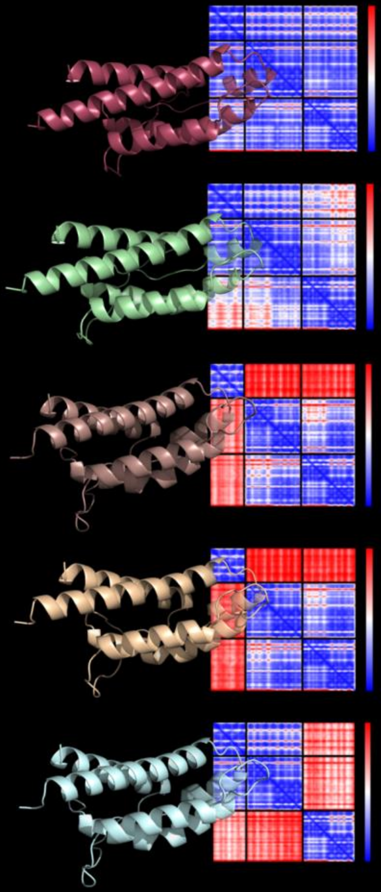
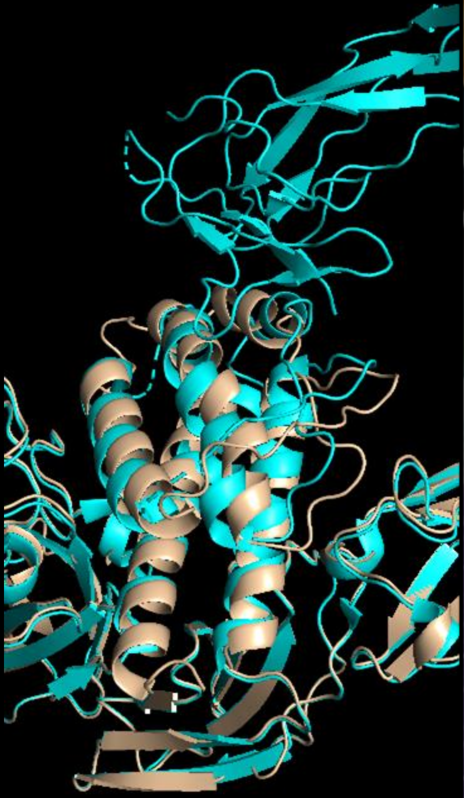
Discovery Studio



ColabFold.adv



ZDOCK, PyMOL



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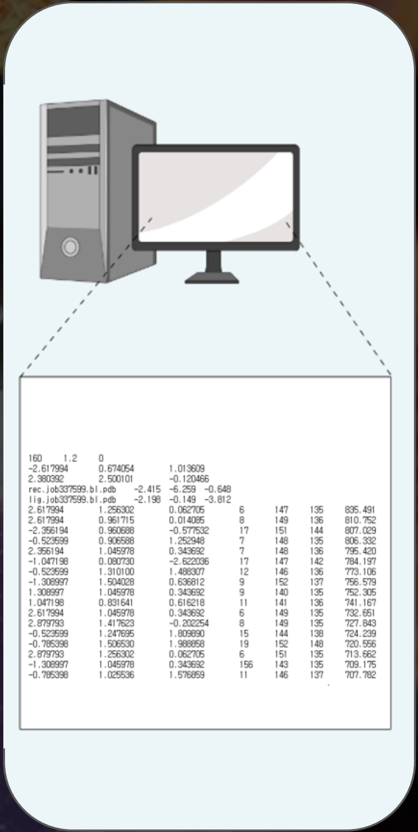

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2.617994   0.961715   0.014085   8    149   136   810.752
-2.356194  0.960688   -0.577532  17   151   144   807.029
-0.523599  0.906588   1.252948   7    148   135   806.332
2.356194   1.045978   0.343692   7    148   136   795.420
-1.047198  0.080730   -2.622036  17   147   142   784.197
-0.523599  1.310100   1.488307   12   146   136   773.106
-1.308997  1.504028   0.636812   9    152   137   756.579
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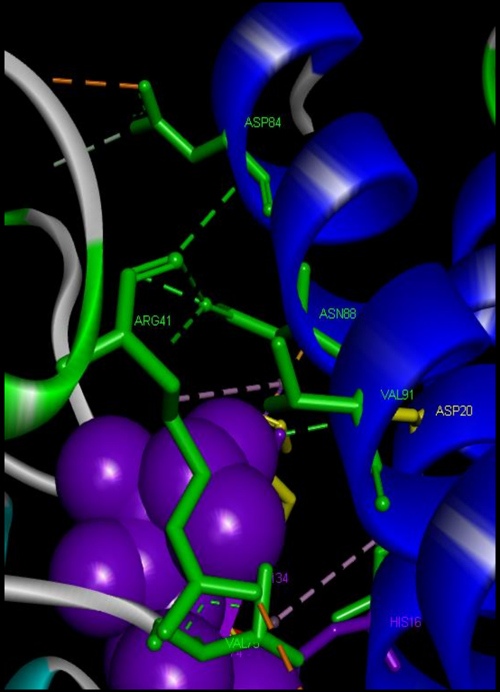
Discovery Studio



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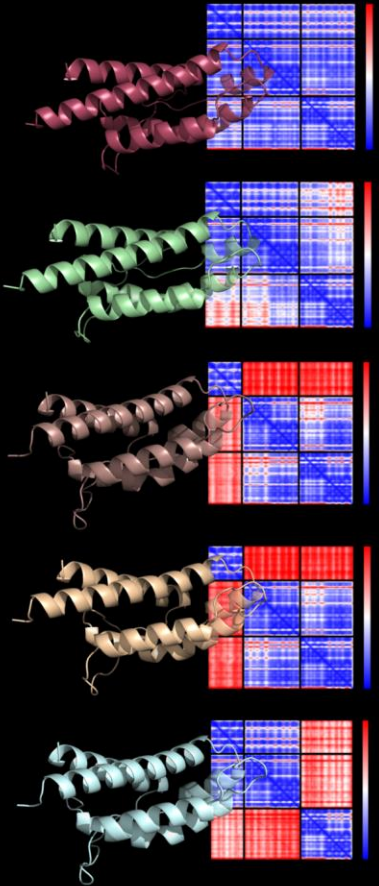
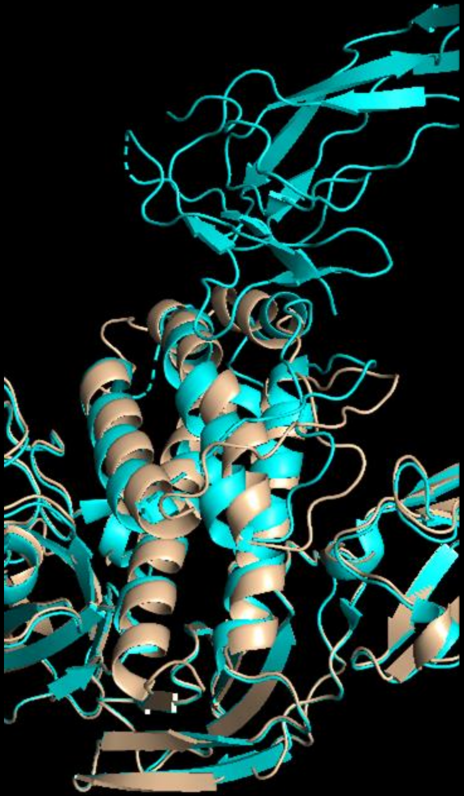


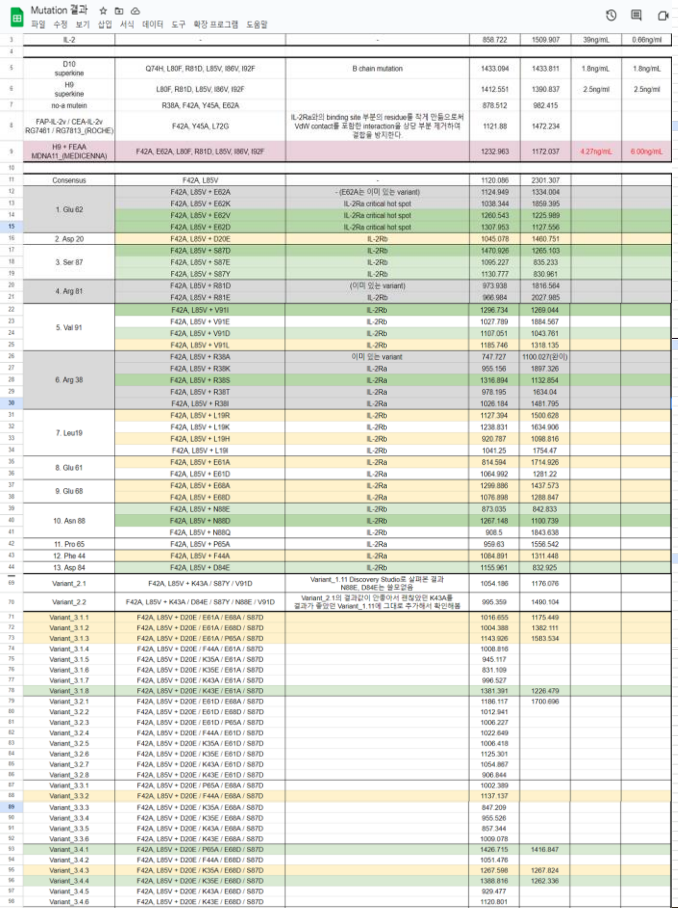
ZDOCK, PyMOL



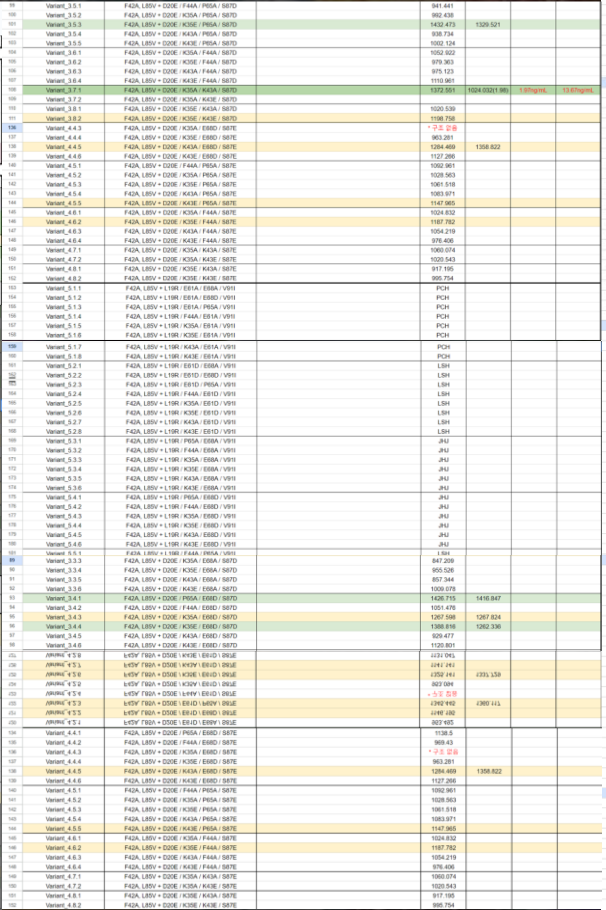
ZDOCK

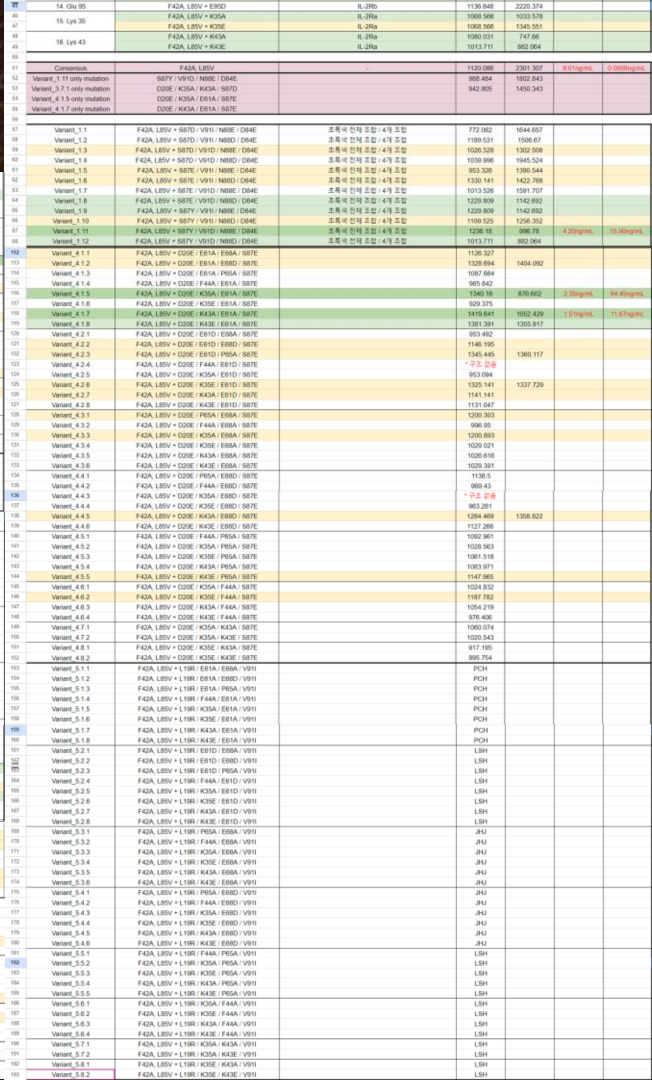
142	$\boxed{1372.547}$
141	912.336
8	819.273
143	817.246
10	769.625
143	764.840
139	761.043
1	746.891
0	712.141
1	693.117
143	673.668
142	650.137
140	640.242
10	623.398
8	622.020

Variant Trial - 197개 시도



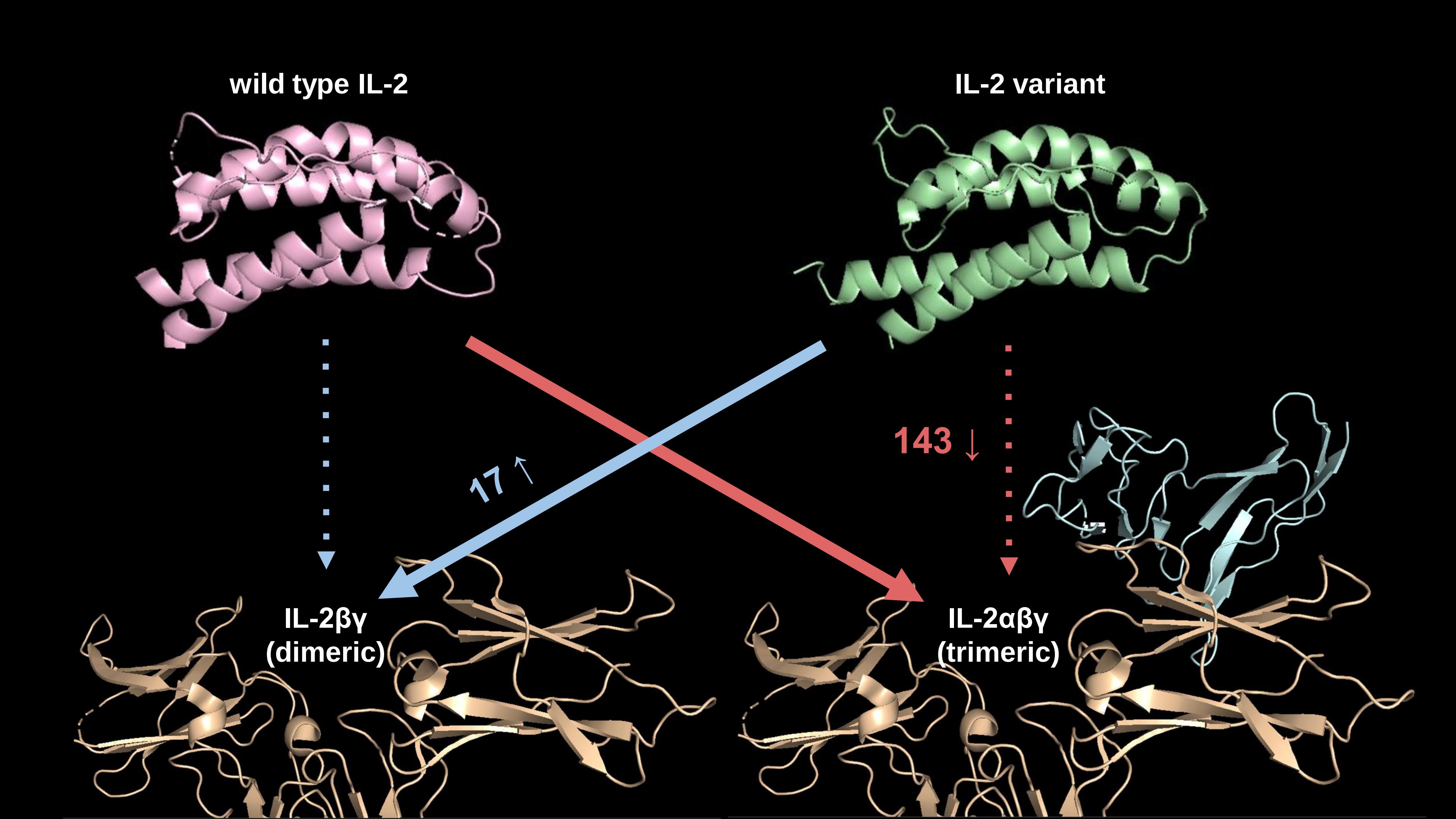
IL-2	-	-	858.722	1509.907	39ng/mL	0.66ng/ml
D10 superkine	Q74H, L80F, R81D, L85V, I86V, I92F	B chain mutation	1433.094	1433.811	1.8ng/mL	1.8ng/mL
H9 superkine	L80F, R81D, L85V, I86V, I92F		1412.551	1390.837	2.5ng/ml	2.5ng/ml
no-α mutein	R38A, F42A, Y45A, E62A		878.512	982.415		
FAP-IL-2v / CEA-IL-2v RG7461 / RG7813 (ROCHE)	F42A, Y45A, L72G	IL-2Rα와의 binding site 부분의 residue를 작게 만듦으로써 VdW contact를 포함한 interaction을 상당 부분 제거하여 결합을 방지한다.	1121.88	1472.234		
H9 + FEAA MDNA11 (MEDICENNA)	F42A, E62A, L80F, R81D, L85V, I86V, I92F		1232.963	1172.037	4.27ng/mL	6.00ng/mL
Consensus	F42A, L85V	-	1120.086	2301.307		
1. Glu 62	F42A, L85V + E62A	- (E62A는 이미 있는 variant)	1124.949	1334.004		
	F42A, L85V + E62K	IL-2Rα critical hot spot	1038.344	1858.395		
	F42A, L85V + E62V	IL-2Rα critical hot spot	1260.543	1225.989		
	F42A, L85V + E62D	IL-2Rα critical hot spot	1307.953	1127.556		
2. Asp 20	F42A, L85V + D20E	IL-2Rb	1045.078	1460.751		
3. Ser 87	F42A, L85V + S87D	IL-2Rb	1470.926	1265.103		
	F42A, L85V + S87E	IL-2Rb	1095.227	835.233		
	F42A, L85V + S87Y	IL-2Rb	1130.777	830.961		
4. Arg 81	F42A, L85V + R81D	(이미 있는 variant)	973.938	1816.564		
	F42A, L85V + R81E	IL-2Rb	966.984	2027.985		
5. Val 91	F42A, L85V + V91I	IL-2Rb	1296.734	1269.044		
	F42A, L85V + V91E	IL-2Rb	1027.789	1884.567		
	F42A, L85V + V91D	IL-2Rb	1107.051	1043.761		
	F42A, L85V + V91L	IL-2Rb	1185.746	1318.135		
6. Arg 38	F42A, L85V + R38A	이미 있는 variant	747.727	1100.027(완이)		
	F42A, L85V + R38K	IL-2Ra	955.156	1897.326		
	F42A, L85V + R38S	IL-2Ra	1316.894	1132.854		
	F42A, L85V + R38T	IL-2Ra	978.195	1634.04		
	F42A, L85V + R38I	IL-2Ra	1026.184	1481.795		
7. Leu19	F42A, L85V + L19R	IL-2Rb	1127.394	1500.628		
	F42A, L85V + L19K	IL-2Rb	1238.831	1634.906		
	F42A, L85V + L19H	IL-2Rb	920.787	1098.816		
	F42A, L85V + L19I	IL-2Rb	1041.25	1754.47		
8. Glu 61	F42A, L85V + E61A	IL-2Ra	814.594	1714.926		
	F42A, L85V + E61D	IL-2Ra	1064.992	1281.22		
9. Glu 68	F42A, L85V + E68A	IL-2Ra	1299.886	1437.573		
	F42A, L85V + E68D	IL-2Ra	1076.898	1288.847		
10. Asn 88	F42A, L85V + N88E	IL-2Rb	873.035	842.833		
	F42A, L85V + N88D	IL-2Rb	1267.148	1100.739		
	F42A, L85V + N88Q	IL-2Rb	908.5	1843.638		
11. Pro 65	F42A, L85V + P65A	IL-2Ra	959.63	1556.542		
12. Phe 44	F42A, L85V + F44A	IL-2Ra	1084.891	1311.448		
13. Asp 84	F42A, L85V + D84E	IL-2Rb	1155.961	832.925		
Variant_2.1	F42A, L85V + K43A / S87Y / V91D	Variant_1.11 Discovery Studio로 살펴본 결과 N88E, D84E는 필요없음	1054.186	1176.076		
Variant_2.2	F42A, L85V + K43A / D84E / S87Y / N88E / V91D	Variant_2.1의 결과값이 안좋아서 괜찮았던 K43A를 결과가 좋았던 Variant_1.11에 그대로 추가해서 확인해봄	995.359	1490.104		
Variant_3.1.1	F42A, L85V + D20E / E61A / E68A / S87D		1016.655	1175.449		
Variant_3.1.2	F42A, L85V + D20E / E61A / E68D / S87D		1004.388	1382.111		
Variant_3.1.3	F42A, L85V + D20E / E61A / P65A / S87D		1143.926	1583.534		
Variant_3.1.4	F42A, L85V + D20E / F44A / E61A / S87D		1008.816			
Variant_3.1.5	F42A, L85V + D20E / K35A / E61A / S87D		945.117			
Variant_3.1.6	F42A, L85V + D20E / K35E / E61A / S87D		831.109			
Variant_3.1.7	F42A, L85V + D20E / K43A / E61A / S87D		996.527			
Variant_3.1.8	F42A, L85V + D20E / K43E / E61A / S87D		1381.391	1226.479		
Variant_3.2.1	F42A, L85V + D20E / E61D / E68A / S87D		1186.117	1700.696		
Variant_3.2.2	F42A, L85V + D20E / E61D / E68D / S87D		1012.941			
Variant_3.2.3	F42A, L85V + D20E / E61D / P65A / S87D		1006.227			
Variant_3.2.4	F42A, L85V + D20E / F44A / E61D / S87D		1022.649			
Variant_3.2.5	F42A, L85V + D20E / K35A / E61D / S87D		1006.418			
Variant_3.2.6	F42A, L85V + D20E / K35E / E61D / S87D		1125.301			
Variant_3.2.7	F42A, L85V + D20E / K43A / E61D / S87D		1054.867			
Variant_3.2.8	F42A, L85V + D20E / K43E / E61D / S87D		906.844			
Variant_3.3.1	F42A, L85V + D20E / P65A / E68A / S87D		1002.389			
Variant_3.3.2	F42A, L85V + D20E / F44A / E68A / S87D		1137.137			
Variant_3.3.3	F42A, L85V + D20E / K35A / E68A / S87D		847.209			
Variant_3.3.4	F42A, L85V + D20E / K35E / E68A / S87D		955.526			
Variant_3.3.5	F42A, L85V + D20E / K43A / E68A / S87D		857.344			
Variant_3.3.6	F42A, L85V + D20E / K43E / E68A / S87D		1009.078			
Variant_3.4.1	F42A, L85V + D20E / P65A / E68D / S87D		1426.715	1416.847		
Variant_3.4.2	F42A, L85V + D20E / F44A / E68D / S87D		1051.476			
Variant_3.4.3	F42A, L85V + D20E / K35A / E68D / S87D		1267.598	1267.824		
Variant_3.4.4	F42A, L85V + D20E / K35E / E68D / S87D		1388.816	1262.336		
Variant_3.4.5	F42A, L85V + D20E / K43A / E68D / S87D		929.477			
Variant_3.4.6	F42A, L85V + D20E / K43E / E68D / S87D		1120.801			





Final Variant

			ZDOCK score		EC50	
		변경 잔기(mutation)	2R (이량체수용체)	3R (삼량체수용체)	2R (이량체수용체)	3R (삼량체수용체)
비교 대상	human IL-2	-	858.722	1509.907	39ng/mL (1배)	0.66ng/mL (1배)
	MDNA11 (MEDICENNA)	F42A, E62A, L80F, R81D, L85V, I86V, I92F	1232.963	1172.037	4.27ng/mL (9배↑)	6.00ng/mL (9배↓)
1세대 변이체	Variant_1.11	F42A, D84E, L85V, S87Y, N88E, V91D	1236.18	996.78	4.20ng/mL (9배↑)	15.90ng/mL (24배↓)
2세대 변이체	Variant_2.7.1	D20E, K35A, F42A, K43A, L85V, S87D	1372.551	1024.032	1.97ng/mL (20배↑)	13.67ng/mL (21배↓)
3세대 변이체	Variant_3.1.5	D20E, K35A, F42A, E61A, L85V, S87E	1340.16	676.602	2.35ng/mL (17배↑)	94.45ng/mL (143배↓)
	Variant_3.1.7	D20E, F42A, K43A, E61A, L85V, S87E	1419.641	1052.429	1.51ng/mL (26배↑)	11.67ng/mL (18배↓)



wild type IL-2
IL-2 variant
17 ↑
143 ↓
IL-2βγ
(dimeric)
IL-2αβγ
(trimeric)

Variant Sequence

구성	서열
MDNA11 (MEDICENNA) (서열번호45)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEALKP LEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL T
V1.11 (서열번호7)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPREVIYE INDIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
V2.7.1 (서열번호18)	APTSSSTKKTQLQLEHLLLELQMILNGINNYKNPALTRMLTAAFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDVIDN INVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
V3.1.5 (서열번호22)	APTSSSTKKTQLQLEHLLLELQMILNGINNYKNPALTRMLTAKFYMPKKATELKHLQCLEAELKP LEEVLNLAQSKNFHLRPRDVIEN INVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
V3.1.7 (서열번호23)	APTSSSTKKTQLQLEHLLLELQMILNGINNYKNPKLTRMLTAAFYMPKKATELKHLQCLEAELKP LEEVLNLAQSKNFHLRPRDVIEN INVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

Provisional Patent

결재	담 당		팀 장		단 장	

발 명 신 고 서

발 명 의 명 칭	국문 :인간 인터루킨-2 변이체 폴리펩티드 및 이의 용도		
	영문 :Human Interleukin-2 Variants and uses of thereof		
발 명 자	성 명	지 분 (%)	소 속
	한글: 김용성 (인) Yong Sung Kim 영문: Yong-Sung Kim	25	분자과학기술학과
	한글: 박채현 박채현 영문: Park Chaehyun	25	응용화학생명공학과
	한글: 이상욱 이상욱 영문: Lee SangUk	25	응용화학생명공학과
	한글: 정현정 정현정 영문: Hyunjung J	25	생명과학과
관 련 연구과제	연구과제(과제번호)		계약기관 지원사업

나중에 서지자료 업데이트 하겠습니다.

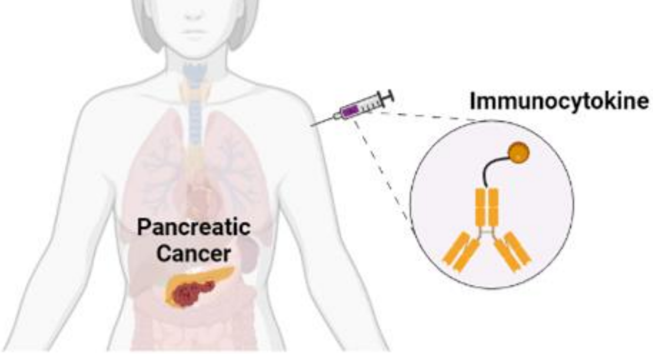
출 원 희 망 국	■ 국내 □ 국외(Korea, Republic of)

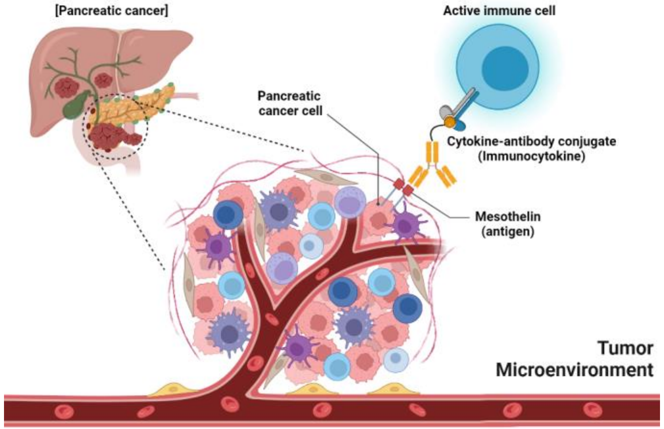
발 명 의 공개여부	공 개 일자	공개구분	내 용

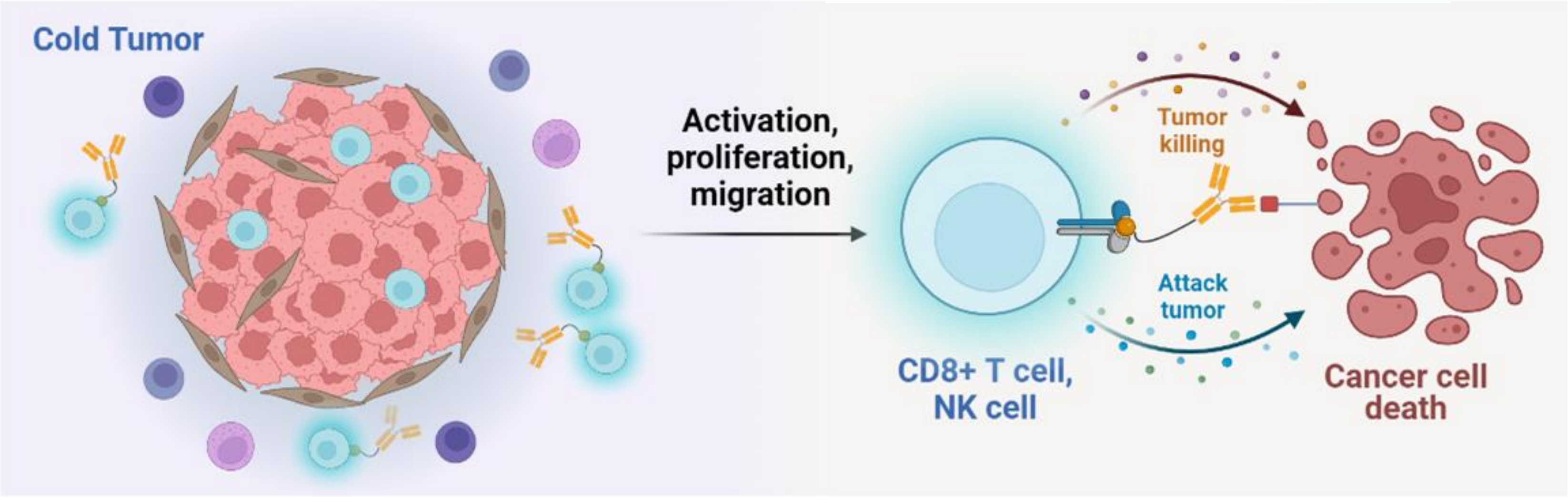
출 원 번 호 통 지 서

출 원 일 자	2023.05.30
특 기 사 항	심사청구(무) 공개신청(무) 참조번호(107)
출 원 번 호	10-2023-0069278 (접수번호 1-1-2023-0595082-19) (DAS접근코드4FEF)
출 원 인 명 칭	아주대학교산학협력단(2-2004-025742-3)
대 리 인 성 명	이처영(9-2003-000118-9)
발 명 자 성 명	김용성 박채현 이상욱 정현정
발 명 의 명 칭	인터루킨2 변이체, 이를 포함하는 융합단백질 및 이의 용도

* Mechanism of Action







감사합니다.