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Research Article

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Time to Change to Time: Single Amino Acid Resolution Anti-Tumor Platform Tag-Tack™ is Standby

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ABSTRACT
In order to shorten drug's evolution time than personal cancer's evolution time, a Tag-Tack™ platform was designed and constructed to make introcytoplastic missense proteins to be druggable. We screened 8 human ScFVs specific targeting for 6 introcytoplastic missense proteins, and then cloned them into the platform to be Tag-Tacker™. 6 cell-lines harboring the former 6 introcytoplastic missense proteins were target killed by those 8 Tag-Tackers™, while no tagging protein cell-lines were alive. Thereby, a single amino acid resolution anti-tumor platform is standby!

Main

We do know there are many tumors specific missense proteins which were failed to be druggable tagets or antigens years ago. Today, we designed and constructed an anti-tumor platform which can make those missense introcytoplasmic proteins druggable. Missense protein specific binding ScFVs were designed to be the Tagging domain. An attacking domain would be released by the protein transforming triggered by ScFV- antigen binding. We name this platform Tag-Tack™ for the Tagging(binding) and Attacking(killing). Here are those Tag-Tacker™'s anti-tumour show.

Materials and Methods

- **ScFVs:** P2, P4, P10 are 3 ScFVs for PIK3CA p.E545K missense protein;
- B1 is a ScFV for BRAF p.V600E missense protein;
- K46 is a ScFV for KRAS p.G13D missense protein;
- I4 is a ScFV for IDH1 p.R132C missense protein;
- A7 is a ScFV for EML4-ALK v3b fused protein;
- T6 is a ScFV for TP53 p.R248Q missense protein;
- All these ScFVs are screened from human phage display antibody banks which was built by our lab.

Tag-tack™ Vector EH: This vector is designed and consructed for human. XhoI and NotI are restriction endonuclease site for cloning ScFV.

Tag-tack™ Vector EM: This vector is designed and consructed for mouse. XhoI and NotI are restriction endonuclease site for cloning ScFV.

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Tag-tacker™s

- P2, P4, P10 cloned into EH are Tag-tacker™s PH2, PH4, PH10 for PIK3CA p.E545K missense protein;
- B1 cloned into EH is Tag-tacker™ BH1 for BRAF p.V600E missense protein;
- K46 cloned into EH is Tag-tacker™ KH46 for KRAS p.G13D missense protein;
- I4 cloned into EH is Tag-tacker™ IH4 for IDH1 p.R132C missense protein;
- A7 cloned into EH is Tag-tacker™ AH7 for EML4-ALK v3a fused protein;
- T6 cloned into EH is Tag-tacker™ TH6 for TP53 p.R248Q missense protein;
- T6 cloned into EM is Tag-tacker™ TM6 for TP53 p.R248Q missense protein;

Cell Lines

- TCCSUP haboring PIK3CA p.E545K missense protein [1];
- T84 haboring KRAS p.G13D missense protein [1];
- HT-29 haboring BRAF p.V600E missense protein [1];
- HT-1080 haboring IDH1 p.R132C missense protein [1];
- H2228 haboring EML4-ALK v3b fused protein [2];
- SK-UT-1 haboring TP53 p.R248Q missense protein [1]

Penicillin-Streptomycin Solution	Shanghai YuanPei Biotech Ltd
Fetal bovine serum (FBS)	Israel Beit Haemek
MEM and 1640 culture	Gibco company
Trypsin-EDTA solution	Shanghai XP Biomed Ltd
Lipofectamine 2000	Invitrogen
Cell Counting Kit-8(CCK-8)	Beyotime Biotechnology
XhoI and NotI Restriction Enzyme	Thermo Scientific

CCK-8 Test

1. Seeding cells in 96 wells plate as setting tables;
2. When the cells are cover 70% well, change the culture into 100ul MEM without FBS.
3. Adding 50ul/well transfecting MIX as setting Groups, and incubation in 37°C, 5% CO2 for 48h;
4. Adding 10ul CCK-8 solution in every 100ul well, and incubating for 2h;
5. Measuring the OD of each well in 450nm.

Statistical Analysis

One-way ANOVA in SPSS 22.0 is adapted to OD data statistical analysis, and $P<0.05$ means significance difference between groups.

Group setting

PIK3CA p.E545K Tag-Tacker™ Setting

Cell Seeding: Diluting TCCSUP (PIK3CA p.E545K positive) and H2228 (PIK3CA p.E545K negative) into 5000 cells/mL, and seeding those cells into 96 wells plate in 100μL/well as table1. Every group seeding 6 wells, A1-8, J1-8, K1-8, L1-8, B1-I1, B8-I8 are blank.

Table 1: TCCSUP (+) and H2228(-) seeding in 96 wells plate

	1	2	3	4	5	6	7	8
A								
B		+	+	+	+	+	+	
C		–	–	–	–	–	–	
D		+	+	+	+	+	+	
E		+	+	+	+	+	+	
F		+	+	+	+	+	+	
G		–	–	–	–	–	–	
H		+	+	+	+	+	+	
I		–	–	–	–	–	–	
J								
K								
L								

Cytotoxicity Test Grouping

When the cells are cover 70% well, change the culture into 100ul MEM without FBS. Adding 50ul/well transfection MIX as table 2.

Table 2: Tag-Tacker™ PH10, PH2, PH4 Transfecting Groups

	1	2	3	4	5	6	7	8
A								
B		PH10+Lipofectamine2000						
C		PH10+Lipofectamine2000						
D		PH10						
E		Lipofectamine2000						
F		PH2+Lipofectamine2000						
G		PH2+Lipofectamine2000						
H		PH4+Lipofectamine2000						
I		PH4+Lipofectamine2000						
J								
K								
L								

BRAF p.V600E Tag-Tacker™ Setting

Cells Seeding

Seeding HT-29 (BRAF p.V600E positive) and H2228 (BRAF p.V600E negative) in 5000 cells/well as table 3, And culturing in 37°C, 5% CO2. A11-12, B11-12, C11-12, D11-12, E1-12, F1-12, G1-12, H1-12 are blank.

Table 3: HT-29(+) and H2228(-) Cells Seeding in 96 wells plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	+	+	+	+	+	+	+	+	+		
B	-	-	-	-	-	-	-	-	-	-		
C	+	+	+	+	+	+	+	+	+	+		
D	+	+	+	+	+	+	+	+	+	+		
E												
F												
G												
H												

Cytotoxicity Test Grouping

When the cells are cover 70% well, change the culture into 100ul MEM without FBS. Adding 50ul/well transfecting MIX as table 4.

Table 4: Tag-Tacker™ BH1 Transfecting Groups

	1	2	3	4	5	6	7	8	9	10	11	12
A	BH1+Lepofectamine 2000											
B	BH1+Lepofectamine 2000											
C	BH1											
D	Lipofectamine 2000											
E												
F												
G												
H												

KRAS p.G13D Tag-Tacker™ Setting

Cell Seeding

Seeding T84 (KRAS p.G13D positive) and HT-29 (KRAS p.G13D negative) in 5000 cells/well as table 5, And culturing in 37°C, 5% CO2. A_{1-12}, $B_{1,6,7,12}$, $C_{1,6,7,12,}$ $D_{1,6,7,12,}$ $E_{1,6,7,12,}$ $F_{1-12,}$ G_{1-12}, H_{1-12} are blank.

Table 5: T84(+) and HT-29(-) Cell Seeding in 96 Wells Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		+	+	+	+			-	-	-	-	
C		+	+	+	+			-	-	-	-	
D		+	+	+	+			-	-	-	-	
E		+	+	+	+			-	-	-	-	
F												
G												
H												

Cytotoxicity Test Grouping

When the cells are cover 70% well, change the culture into 100ul MEM without FBS. Adding 50ul/well transfecting MIX as table 6.

Table 6: Tag-Tacker™ KH46 Transfecting Groups

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B1: KH46+Lipo2000						B2: KH46+Lipo2000				
C		C1: KH46						C2: KH46				
D		D1: Lipo2000						D2: Lipo2000				
E		E1: MEM culture						E2: MEM culture				
F												
G												
H												

IDH1 p.R132C Tag-Tacker™ Setting

Cell Seeding

Seeding HT-1080 cells (IDH1 p.R132C positive) and HCT-116 cells (IDH1 p.R132C negative) in 5000 cells/well as table 7, And culturing in 37°C, 5% CO2. $A_{1-12,}$ $B_{1,6,7,12,}$ $C_{1,6,7,12,}$ $D_{1,6,7,12,}$ $E_{1,6,7,12,}$ $F_{1-12,}$ $G_{1-12,}$ H_{1-12} are blank.

Table 7: HT-1080(+) and HCT-116(-) Seeding in 96 Wells Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		+	+	+	+			-	-	-	-	
C		+	+	+	+			-	-	-	-	
D		+	+	+	+			-	-	-	-	
E		+	+	+	+			-	-	-	-	
F												
G												
H												

Cytotoxicity Test Grouping

When the cells are cover 70% well, change the culture into 100ul MEM without FBS. Adding 50ul/well transfecting MIX as table 8.

Table 8: Tag-Tacker™ IH4 Transfecting Gruops

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B1:IH4+Lipo2000						B2:IH4+Lipo2000				
C		C1: lipo2000						C2: lipo2000				
D		D1: IH4						D2: IH4				
E		E1: MEM culture						E2: MEM culture				
F												
G												
H												

EML4-ALK v3a Tag-Tacker™ Setting

Cell Seeding and Grouping

Seeding H2228 cells (EML4-ALK v3b positive) and HCT-116 cells (EML4-ALK v3b negative) in 5000 cells/well as table 9, And culturing in 37°C, 5% CO2. $A_{1-12,}$ $B_{1,6,7,12,}$ $C_{1,6,7,12,}$ $D_{1,6,7,12,}$ $E_{1,6,7,12,}$ $F_{1-12,}$ $G_{1-12,}$ H_{1-12} are blank.

Table 9: H2228(+) and HCT-116(-) Seeding in 96 Wells Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		+	+	+	+			-	-	-	-	
C		+	+	+	+			-	-	-	-	
D		+	+	+	+			-	-	-	-	
E		+	+	+	+			-	-	-	-	
F												
G												
H												

Cytotoxicity Test Grouping

When the cells are cover 70% well, change the culture into 100ul MEM without FBS. Adding 50ul/well transfecting MIX as table 10.

Table 10: Tag-Tacker™ AH7 Transfecting Group

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B1:AH7+Lipo2000						B2: H7+Lipo2000				

C		C1: lipo2000						C2: lipo2000				
D		D1: AH7						D2: AH7				
E		E1: MEM culture						E2: MEM culture				
F												
G												
H												

TP53 p.R248Q Tag-TackerTM Setting

Cell Seeding and Grouping

Seeding SK-UT-1 cells (TP53 p.R248Q positive) and T47D cells (TP53 p.R248Q negative) in 5000 cells/well as table 11, And culturing in 37℃,5% CO2. $A_{1\text{-}12,}$ $B_{1,6,7,12,}$ $C_{1,6,7,12,}$ $D_{1,6,7,12,}$ $E_{1,6,7,12,}$ $F_{1\text{-}12,}$ $G_{1\text{-}12,}$ $H_{1\text{-}12}$ are blank.

Table 11: SK-UT-1(+) and T47D (-) Seeding in 96 Wells Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		+	+	+	+			-	-	-	-	
C		+	+	+	+			-	-	-	-	
D		+	+	+	+			-	-	-	-	
E		+	+	+	+			-	-	-	-	
F												
G												
H												

Cytotoxicity Test Grouping

When the cells are cover 70% well, change the culture into 100ul MEM without FBS. Adding 50ul/well transfecting MIX as table 12.

Table 12: Tag-Tacker™ TH6 Transfecting Group

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B1:TH7+Lipo2000						B2TH7+Lipo2000				
C		C1: lipo2000						C2: lipo2000				
D		D1: TH7						D2: TH7				
E		E1: MEM culture						E2: MEM culture				
F												
G												
H												

Results

PIK3CA p.E545K Tag-Tacker™ Results

Cell morphology before and after transfecting PH10

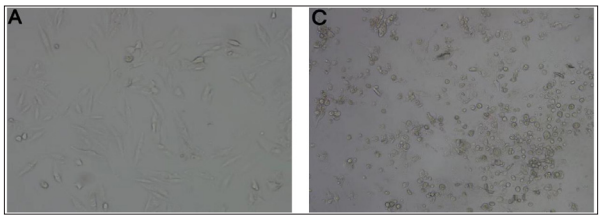

Figure 1: TCCSUP (Group B) before (Figure A) and after (Figure C) PH10 transfecting (10×)

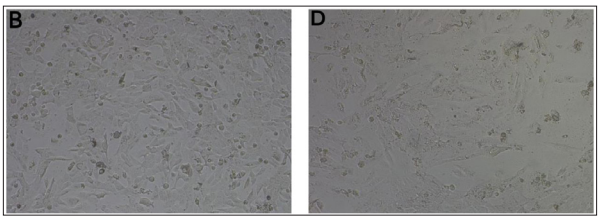

Figure 2: H2228 (Group C) before (Figure B) and after (Figure D) PH10 transfecting (10×)

OD450 Results of the CCK-8 Test After Transfecting Tag-Tacker™ PH10, PH2,PH4:

Table 13: OD in 450nm after transfecting Tag-Tacker™ PH10, PH2, PH4

	1	2	3	4	5	6	7	8
A								
B		0.006	0.009	0.011	0.019	0.021	0.020	
C		0.011	0.146	0.088	0.033	0.044	0.045	
D		0.088	0.044	0.060	0.056	0.067	0.049	
E		0.033	0.055	0.045	0.069	0.152	0.088	
F		0.025	0.018	0.021	0.050	0.035	0.035	
G		0.008	0.165	0.039	0.166	0.088	0.058	
H		0.012	0.017	0.024	0.029	0.029	0.033	
I		0.054	0.205	0.068	0.138	0.027	0.037	
J								
K								
L								

Table 14: Statistic Results of Tag-Tacker™ PH10, PH2,PH4 ($\bar{x}$±s)

Groups	$\bar{x}$±s	P value between groups		
B	0.014±0.006			
C	0.061±0.049	0.025 (B with C)		
D	0.061±0.016	0.026 (B with D)	0.983 (C with D)	
E	0.074±0.043	0.006 (B with E)	0.606 (C with E)	0.591 (D with E)
F	0.031±0.012			
G	0.087±0.066	0.023 (F with G)		
H	0.024±0.008			
I	0.088±0.069	0.011 (H with I)		

Most TCCSUP (PIK3CA p.E545K positive) cells were killed after PH10 transfecting (Figure 1 C) while the H2228 (PIK3CA p.E545K nagitive) were alive after PH10 transfecting (Figure 2 D). From the table 14, OD 450nm of group B is significant difference with group D(*P=0.026*) E(*P=0.006*), which means PIK3CA Tag-tacker™ PH10 transfected into TCCSUP killed those PIK3CA p.E545K positive cells: **BINDING TRIGER KILLING.** While group D is not significant difference(*P=0.591*) with group E, which means that without PIK3CA Tag-tacker™ PH10 transfecting into TCCSUP will not kill those positive cells. The most important is that the significant difference(P=0.025) between group B and C, which means even with PIK3CA Tag-

tackerTM PH10 transfected into H2228, there is no cell killing at H2228 for its's PIK3CA p.E545K nagitive: **NO BINDING NO KILLING.** The significant diffence (*P=0.023*) between group F and G mean that PIK3CA Tag-tackerTM PH2 cannot kill H2228 but TCCSUP. It is the same significant difference(*P=0.011*) as group H and I for PIK3CA Tag-tacker™ PH4.

BRAF p.V600E Tag-Tacker™ Results

Cell Morphology Before and After Transfecting Tag-Tacker™ BH1

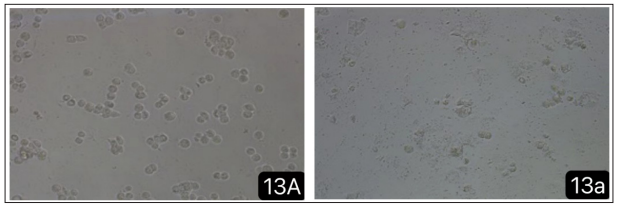

Figure 3: HT-29(Group A) before(13A) and after(13a) BH1 transfecting (20×)

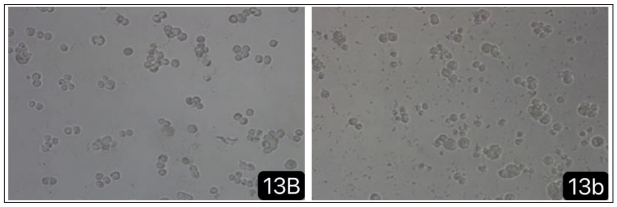

Figure 4: HT-29 (Group C) before(13B) and after(13b) BH1 transfecting (20×)

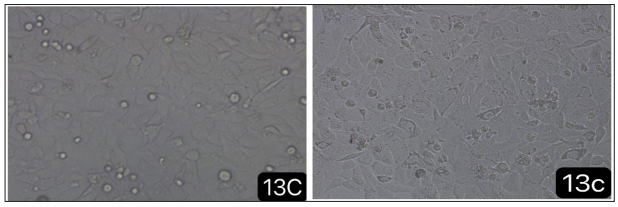

Figure 5: H2228(Group B) before(13C) and after(13c) BH1 tranfecting (20×)

OD450 Results of the CCK-8 Test After Tag-Tacker™ BH1 Transfecting

Table 15: OD450 Results after Transfecting Tag-Tacker™ BH1

	1	2	3	4	5	6	7	8	9	10
A	0.012	0.034	0.005	0.003	0.026	0.019	0.008	0.008	0.012	0.008
B	0.051	0.034	0.100	0.100	0.172	0.059	0.055	0.068	0.166	0.193
C	0.118	0.303	0.580	0.473	0.431	0.341	0.492	0.295	0.718	0.148
D	0.071	0.265	0.479	0.470	0.708	0.193	0.278	0.352	0.274	0.130
E										
F										
G										
H										

Table 16: Statistic Results of Tag-Tacker BH1(x ±s)

Group	x±s	P value between groups	
A	0.014±0.010		
B	0.100±0.057	0.000 (A with B)	
C	0.390±0.187	0.000 (A with C)	
D	0.322±0.189	0.000 (A with D)	0.267 (C with D)

HT-29(BRAF p.V600E positive) in group A were killed after BRAF p.V600E Tag-tackerTM BH1 transfecting (Figure 3), while group C HT-29 were alive without BRAF p.V600E Tag-tackerTM BH1 transfecting (Figure 4). Figure 5 shows that H2228(BRAF p.V600E nagitive) were alive after BRAF p.V600E Tag-tacker™ BH1 transfecting. OD450 statistical analysis results in table 16 shows: The significant difference (P=0.000) between group A and B means that transfecting BRAF p.V600E Tag-tacker™ BH1 do not kill H2228(BRAF p.V600E nagitive) but HT-29(BRAF p.V600E positive): B**INDING TRIGER KILLING.** The significant difference between gouop A and C(*P=0.000)*, D(*P=0.000*) means Tag-tackerTM BH1 works only being transfected into BRAF p.V600E positive cells. Cells are alive with no difference (*P=0.267*) between group C and D: NO BINDING NO KILLING.

KRAS p.G13D Tag-Tacker™ Results

The Cell Figures Before and After Transfecting Tag-TackerTM KH46

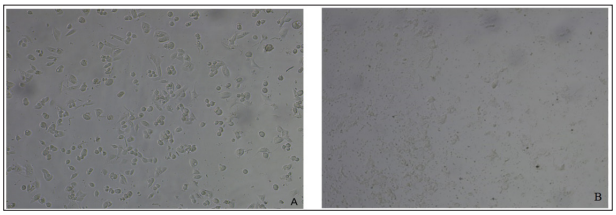

Figure 6: T84(Group B1) before (Figure A) and after Figure B) KH46 transfecting (10×)

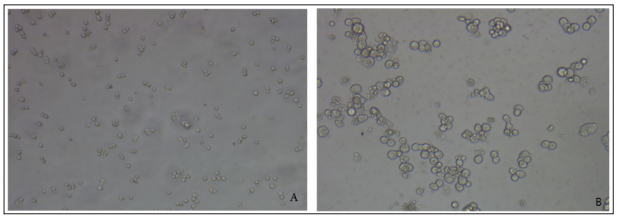

Figure 7: HT-29(Group B2) before (Figure A) and after (Figure B) KH46 transfecting (10×)

OD450 Results of the CCK-8 Test after Tag-Tacker™ KH46 Transfecting

Table 17: OD450 Results After Tag-Tacker™ KH46 Transfecting

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.132	0.214	0.212	0.395			0.421	0.325	0.413	0.452	
C		0.550	0.423	0.562	0.583			0.428	0.521	0.395	0.324	
D		0.349	0.541	0.423	0.352			0.032	0.423	0.485	0.531	
E		0.356	0.464	0.352	0.451			0.032	0.214	0.472	0.523	
F												

G												
H												

Table 18: Statistic Results of Tag-Tacker™ KH46 Transfected T84 (x ±s)

GROUPS	x±S	P value between groups		
B1	0.238±0.111	——		
C1	0.530±0.072	0.000 (B1 with C1)		
D1	0.416±0.090	0.005 (B1 with D1)	0.055(C1 with D1)	
E1	0.406±0.060	0.008 (B1 with E1)	0.038(C1 with E1)	0.849(D1 with E1)

Table 19: Statistic Results of Tag-Tacker™ KH46 Transfected HT-29 (x ±s)

GROUPS	x±S	P value between groups		
B2	0.403±0.054			
C2	0.417±0.082	0.896 (B2 with C2)		
D2	0.368±0.228	0.748 (B2 with D2)	0.652(C2 with D2)	
E2	0.310±0.230	0.401 (B2 with E2)	0.334(C2 with E2)	0.599(D2 with E2)

Most T84 cells (KRAS p.G13D positive) were killed after Tag-tacker™ KH46 transfecting (Figure 6), while HT-29 cells (KRAS p.G13D nagitive) were alive (Figure 7). The OD450 of group B1 is significant difference with C1(*P=0.000*), D1(*P=0.005*), E1(*P=0.008*0, which means KH46 inside cells can kill KRAS p.G13D positive cells: **BINDING TRIGER KILLING.** Group C1 is not significant with D1(P=0.055), and D1 is not with E1(P=0.849), which means KH46 outside cells cannot kill even KRAS p.G13D positive cells. Group B2 is no difference with C2(*P=0.896*), D2(*P=0.748*), E2(*P=0.401*), which means KH46 will not kill KRAS p.G13D nagitive cells inside or outside cells: **NO BINDING NO KILLING.**

IDH1 p.R132C Tag-Tacker™ Results

The Cell Figures Before and After Transfecting Tag-Tacker™ IH4

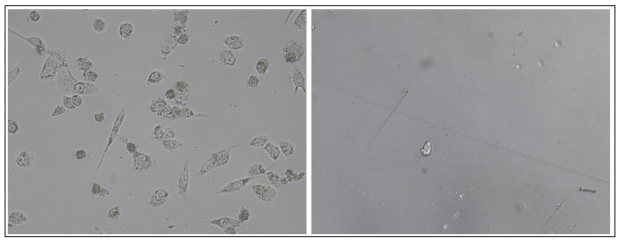

Figure 8: HT-1080(Group B1) before (Figure R) and after (Figure L) IH4 transfecting (20×)

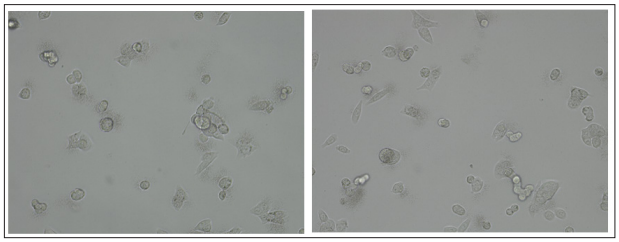

Figure 9: HCT-116(Group B2) before (Figure R) and after (Figure L) IH4 transfecting (20×)

OD450 Results of the CCK-8 Test After Tag-Tacker™ IH4 Transfecting

Table 20: OD450 Results After Tag-Tacker™ IH4 Transfecting

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.003	0.001	0.010	0.006			0.017	0.045	0.013	0.015	
C		0.018	0.018	0.019	0.016			0.025	0.018	0.023	0.017	
D		0.015	0.019	0.013	0.021			0.017	0.015	0.029	0.023	
E		0.026	0.019	0.016	0.017			0.032	0.018	0.036	0.012	
F												
G												
H												

Table 21: Statistic Analysis of IH4 Tag-Tacker™ Transfected HT-1080

GROUPS	x±S	P value between groups		
B2	0.005±0.004	——		
C2	0.018±0.001	0.000(B1 with C1)		
D2	0.017±0.004	0.001(B1 with D1)	0.763(C1 with D1)	
E2	0.020±0.005	0.001(B1 with E1)	0.485(C1 with E1)	0.323(D1 with E1)

Table 22: Statistic Analysis of IH4 Tag-Tacker™ Transfected HCT-116

GROUPS	x±S	P value between groups		
B2	0.023±0.015			
C2	0.021±0.004	0.796(B2 with C2)		
D2	0.021±0.006	0.825(B2 with D2)	0.971(C2 with D2)	
E2	0.025±0.011	0.768(B2 with E2)	0.581(C2 with E2)	0.607(D2 with E2)

Most HT-1080 cells (IDH1 p.R132C positive) were killed after Tag-tacker™ IH4 transfecting (Figure 8), while HCT-116 cells (IDH1 p.R132C nagitive) were alive (Figure 9). The OD450 of group B1 is significant difference with C1(*P=0.000*), D1(*P=0.001*), E1(*P=0.001*), which means IH4 inside cells can kill IDH1 p.R132C positive cells: **BINDING TRIGER KILLING.** Group C1 is not significant with D1(*P=0.763*) and E1(*P=0.485*), while D1 is not with E1(*P=0.323*), which means IH4 outside cells cannot kill even IDH1 p.R132C positive cells. Group B2 is no difference with C2(*P=0.796*), D2(*P=0.825*), E2(*P=0.768*), which means IH4 will not kill IDH1 p.R132C nagitive cells inside or outside cells: **NO BINDING NO KILLING.**

EML4-ALK v3b Tag-Tacker™ Results

The Cell Figures Before and After Transfecting Tag-Tacker™ AH7

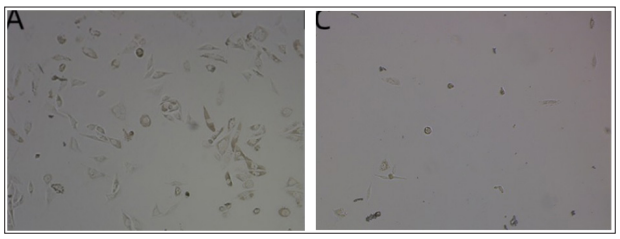

Figure 10: H2228(Group B1) before (Figure A) and after (Figure C) AH7 transfecting (10×)

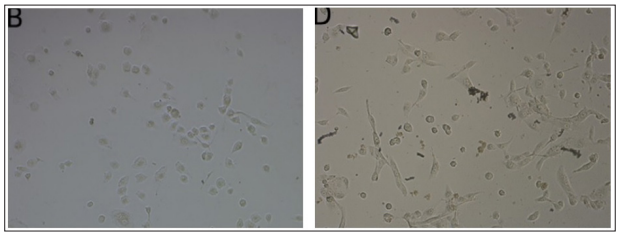

Figure 11: HT-29(Group B2) before (Figure B) and after (Figure D) AH7 transfecting (10×)

OD450 Results of the CCK-8 Test After Tag-Tacker™ AH7 Transfecting

Table 23: OD450 Results After Tag-Tacker™ AH7 Transfecting

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.684	0.681	0.698	0.679			0.414	0.427	0.420	0.405	
C		1.542	1.598	1.523	1.523			0.447	0.445	0.393	0.491	
D		1.512	1.608	1.524	1.532			0.421	0.465	0.435	0.431	
E		1.489	1.591	1.594	1.598			0.395	0.458	0.452	0.423	
F												
G												
H												

Table 24: Statistic Analysis of AH7 Tag-Tacker™ Transfected H2228

GROUPS	*x*±S	P value between groups		
B2	0.686±0.009	—		
C2	1.547±0.035	0.000 (B1 with C1)		
D2	1.544±0.043	0.000 (B1 with D1)	0.920 (C1 with D1)	
E2	1.568±0.053	0.000 (B1 with E1)	0.394 (C1 with E1)	0.343 (D1 with E1)

Table 25: Statistic Analysis of AH7 Tag-Tacker™ Transfected HT-29

GROUPS	*x*±S	P value between groups		
B2	0.417±0.009			
C2	0.444±0.040	0.127 (B2 with C2)		
D2	0.438±0.019	0.226 (B2 with D2)	0.729 (C2 with D2)	
E2	0.432±0.029	0.377 (B2 with E2)	0.492 (C2 with E2)	0.729 (D2 with E2)

Most H2228 cells (EML4-ALK v3b positive) were killed after Tag-tacker™ AH7 transfecting (Figure 10), while HT-29 cells (EML4-ALK v3b nagitive) were alive (Figure 11). The OD450 of group B1 is significant difference with C1(*P=0.000*), D1(*P=0.000*), E1(*P=0.000*), which means AH7 inside cells can kill EML4-ALK v3b positive cells: **BINDING TRIGER KILLING.** Group C1 is not significant with D1(*P=0.920*) and E1(*P=0.394*), while D1 is not with E1(*P=0.343*), which means AH7 outside cells cannot kill even EML4-ALK v3b positive cells. Group B2 is no difference with C2(*P=0.127*), D2(*P=0.226*), E2(*P=0.377*), which means AH7 will not kill EML4-ALK v3b nagitive cells inside or outside cells: **NO BINDING NO KILLING.**

TP53 p.R248Q Tag-Tacker™ Results

The Cell Figures Before and After Transfecting Tag-Tacker™ TH6

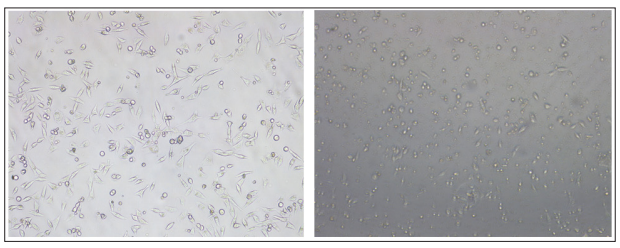

Figure 12: SK-UT-1(Group B1) before (Figure left) and after (Figure right) TH6 transfecting (10×)

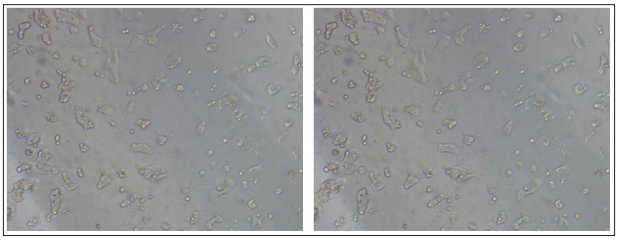

Figure 13: T47D (Group B2) before (Figure left) and after (Figure right) TH6 transfecting (10×)

\OD450 Results of the CCK-8 Test After Tag-Tacker™ TH6 Transfecting

Table 26: OD450 Results After Tag-Tacker™ TH6 Transfecting

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.4869	0.4848	0.4736	0.5108			0.2429	0.2515	0.2427	0.2496	
C		1.3042	1.2582	1.2772	1.2539			0.2499	0.2503	0.2662	0.2756	
D		1.2255	1.1184	1.1211	1.1880			0.2741	0.2647	0.2739	0.2706	
E		0.8336	0.8986	1.0122	1.1281			0.2605	0.2437	0.2483	0.2601	
F												
G												
H												

Table 27: Statistic Analysis of TH6 Tag-Tacker™ Transfected SK-UT-1

GROUPS	*x*±S	P value between groups		
B2	0.4890`±0.016	——		
C2	1.2733±0.023	0.000 (B1 with C1)		
D2	1.1632±0.053	0.000 (B1 with D1)	0.126 (C1 with D1)	
E2	0.9681±0.130	0.000 (B1 with E1)	0.101 (C1 with E1)	0.303 (D1 with E1)

Table 28: Statistic Analysis of TH6 Tag-Tacker™ Transfected T47D

GROUPS	*x*±S	P value between groups		
B2	0.2467±0.005			
C2	0.2605±0.013	0.542 (B2 with C2)		
D2	0.2708±0.004	0.003 (B2 with D2)	0.763 (C2 with D2)	
E2	0.2532±0.008	0.848 (B2 with E2)	0.962 (C2 with E2)	0.128 (D2 with E2)

Most SK-UT-1 cells (TP53 p.R248Q positive) were killed after Tag-tacker™ TH6 transfecting (Figure 12), while T47D cells (TP53 p.R248Q nagitive) were alive (Figure 13). The OD450 of group B1 is significant difference with C1(*P=0.000*), D1(*P=0.000*), E1(*P=0.000*), which means TH6 inside cells can kill TP53 p.R248Q positive cells: **BINDING TRIGER KILLING.** Group C1 is not significant with D1(*P=0.126*) and E1(*P=0.101*), while D1 is not with E1(*P=0.303*), which means TH6 outside cells cannot kill even TP53 p.R248Q positive cells. Group B2 is no difference with C2(*P=0.542*), E2(*P=0.848*), The significance difference between Group B2 and D2(*P=0.003*) is statistic result only, but those cells are live well without any interfere by adding mix (OD-D2:0.2708>OD-B2:0.2467), which means TH6 cannot kill TP53 p.R248Q nagitive cells inside or outside cells: **NO BINDING NO KILLING.**

From those results above, we see:

- ScFVs for PIK3CA p.E545K cloned into Tag-tack™ do kill TCCSUP (PIK3CA p.E545K +) but H2228(PIK3CA p.E545K -): PIK3CA could be tagged!
- ScFV for KRAS p.G13D cloned into Tag-tack™ do kill T84 (KRAS p.G13D +) but HT-29 (KRAS p.G13D -): KRAS could be tagged!
- ScFV for BRAF p.V600E cloned into Tag-tack do kill HT-29 (BRAF p.V600E +) but H2228 (BRAF p.V600E -): BRAF could be tagged!
- ScFV for IDH1 p.R132C cloned into Tag-tack™ do kill HT-1080 (IDH1 p.R132C +) but HCT-116 (IDH1 p.R132C -): IDH1 could be tagged!
- ScFV for EML4-ALK v3a fused protein cloned into Tag-tack™ do kill H2228 (EML4-ALK v3a +) but HCT-116 (EML4-ALK v3a -): ALK fused proteins could be tagged!
- ScFV for TP53 p.R248Q cloned into Tag-tack™ do kill SK-UT-1 (TP53 p.R248Q +) but HCT-116 (TP53 p.R248Q -): TP53 could be tagged!
- So, Tag-tack™ platform do not interfere those cloned ScFVs's target binding!
- And, cloned ScFVs's target binding do triger positive cell killing but nagitive!
- BINDING TRIGER KILLING + NO BINDING NO KILLING = BINDING IS KILLING!
- So, killing is simplified into binding by Tag-tack™!

Discussion

For metastasis cancer, Drug Resistance cause the death of patients. From the progress free survival (PFS), we find the drug resistance cycle is 6 months of Chemo drugs, 10-20 months of TKIs, while a new Drug research cycle is more than 10 years

[3-6]. In one cancer patient's treatment period, cancer cells' evolution is far quick than Drugs' evolution. There is no in-time-drug to stop death!

In order to get in-time-drug in 6 months, we designed a Tag-tack™ platform which could target to almost any missense protein in cytoplasm. Two domains are designed: 1. Tagging domain: A single-chain antibody binding missense protein, which is in charge of specificity binding function; 2. Attacking domain: A covered domain in charge of killing function will be exposed by the structure transforming triggered by ScFV-antigen binding of tagging domain. And those former data confirmed that: TP53, KRAS, PIK3CA, IDH1, BRAF and ALK fused proteins can be tagged! And tagging does triger KILLING! Every cancer has its missense proteins. By the NGS tech, we can get any cancer's missense protein information in one week. By the phage display technique, we can get any missense protein specific ScFV in 3 months or much less (If alphafold 3 is in). 1 week for cloning ScFV into Tag-tack™ platform. A new specific Tag-tacker™ can be produced less than 4 months. On the other hand, we do have the hot missense protein profile of cancers [7,8]. Nowadays, we can almost get any target protein's specific binding ScFV easily and QUICKLY! Thousands of ScFV can be screened before somebody's cancer missense protein information cleared. Thousands of Tag-tacker™s covering every hot missense protein are waiting at cancer's evolution endpoint.

- Today, we can know any cancer's specific missense proteins!
- Today, we can get any missense protein's ScFV!
- With the Tag-tack™, any ScFV-missense protein's BINDING is KILLING!
- By the Tag-tack™, drug is faster than Cancer!
- For the Tag-tack™, Cancer is done!

Author Contribution

Jun Bai invited Tag-tack and Yuan Zhang designed part of this trial; Shiyuan Chen, Wenxuan Xiao, Xuemin Xia, Tong Li built the human phage display antibody bank; Shangzhen Yang, Xincheng He, Shi Tang, Siming Chen, Xiangqian Quan, Haocong Liu, Jia Pei, Lanying Duan,Yang Sun, Yuan Zhang screened those ScFVs, cloned Tag-tacker and tested Tag-tacker's efficacy.

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