# Current status and evolution of immunotherapy for gastrointestinal cancer

**Shoichi Hazama[1], Koji Tamada[2]**, Masao Nakajima[3], Satoshi Matsukuma[3], Yoshitaro Shindo[3], Hiroto Matsui[3], Shinsuke Kanekiyo[3], Iida Michihisa[3], Nobuaki Suzuki[3], Shigefumi Yoshino[4], **Shun Doi[5], Hiroaki Nagano[3]**

Yamaguchi University School of Medicine,
[1]TR and Developmental Therapeutics against Cancer, [2]Department of immunology,
[3]Gastroenterological, Breast and Endocrine Surgery, [4]Oncology center.
[5]CYTLIMIC Inc.

Although immune-checkpoint inhibitors (ICI) have revealed that patients already carry the neoantigens specific immunity (Neo-CTL), the response rate is still remain within 0-30% for gastrointestinal (GI) cancers. **What we should do next are, detecting immunosuppressive biomarker and overcoming the immunosuppression by novel immune-modifiers, and enhance Neo-CTL.**

**Cancer Immunotherapy**
**Breakthrough of the Year for 2013**


**CTLA-4 was discovered in 1987.**
**In 1996, Allison published a paper in *Science* showing that antibodies against CTLA-4 erased tumors in mice.**

**In 1999, it acquired rights to the antibody, taking the leap from biology to drug.**

**The numbers for another antibody are so far even better and the side effects milder. In the early 1990s, a biologist in Japan, Tasuku Honjo discovered a molecule expressed in dying T cells, which he called programmed death 1, or PD-1, and which he recognized as another brake on T cells.**

**One, oncologist Drew Pardoll at Johns Hopkins University, met with a leader of Medarex at a Baltimore coffee shop. He urged the company to test an anti–PD-1 antibody in people.**

**The first trial, with 39 patients and five different cancers, began in 2006.**

SCIENCE VOL 342 20 DECEMBER 2013

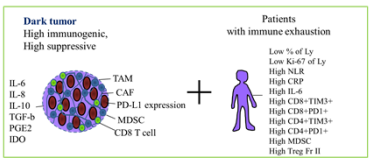
**Published Study of immune checkpoint inhibitors for gastrointestinal cancers**

| Tumor Type | Target | Key Drug and Trial Identifier | Treatment Line | Phase | Allocation | Sample Size | Clinical Efficacy | irAE |
|---|---|---|---|---|---|---|---|---|
| ESCC | PD-1 | Nivolumab, ONO-4538-07 | Late | II | Single arm | 64 | ORR, 11 of 64 (17%) | lung infection (4), dehydration (2), IP (2) of 65. No treatment-related deaths. |
| GC or GEJC | PD-1 | Nivolumab, ATTRACTION-2 | 3rd or more | III | randomized, double-blind | 493 (330 vs 163) | ORR, 30 (11·2%) of 268, 1Y OS: 26.2% (Nivo) vs 10.9% (Place) | Grade3 or 4 irAE in 34 (10%); irAE led to death in 5 (2%). |
| PD-L1+ GC or GEJC | PD-1 | Pembrolizumab, KEYNOTE-012 | Late | 1b | Single arm | 39 | ORR, 22% (8 of 36) | Grade3, 2 fatigue, 1 PG, 1 hypothyroidism, 1 PSN; Grade4, 1 IP. No treatment-related deaths. |
| GC or GEJC | PD-1 | Pembrolizumab, KEYNOTE-059-Cohort 1 | 3rd or more | II | Single arm | 259 | ORR, 15.5% in PD-L1+ pts, 5.5% in PD-L1− pts. | Grade3-5 irAE in 43 (16.6%), discontinuation in 2 (LFT, BDS), fatal in 2 (AKI, PE). |
| dMMR/MSI-H CRC | PD-1 | Pembrolizumab, CT01876511 | Late | II | Single arm | 28 | ORR, 40% (4 of 10) for dMMR/MSI-H CRC, 0% (0 of 18) for pMMR CRC | Grade3 and 4 irAE, anemia (17%), lymphopenia (20%), diarrhea (5%), BO, (7%) |
| dMMR/MSI-H tumors | PD-1 | Pembrolizumab, CT01876511 | 2nd or more | II | Single arm | 86 | ORR, 53%; CR, 21% | irAEs were manageable. 74% had AE (grade1 or more), hypothyroidism (21%) managed with THR. |
| dMMR/MSI-H CRC | PD-1 | Nivolumab, CheckMate 142 | 2nd or more | II | Single arm | 74 | ORR, 31.1% (23 of 74) | Common grade 3 or 4 irAE, elevation of lipase (6) and amylase (2). No treatment-related deaths. |
| dMMR/MSI-H CRC | PD-1 CTLA-4 | Nivolumab + Ipilimumab CheckMate 142 | 2nd or more | II | Single arm | 119 | ORR, 55% (65 of 119), 4 CR, 61 PR | Grade 3 irAE in 32, AST or ALT (11%), lipase (4%), anemia or colitis (3%), hypothyroidism (1%). No treatment-related deaths. |
| HCC | PD-1 | Nivolumab, CheckMate 040 | 1st or more | I/II | Dose escalation and expansion | P I, 48 P II, 214 | ORR, 15% (3CR, 4PR of 48) in P I, 20% in P II (3CR, 39PR of 214) | 12 (25%) of 48 had grade 3/4 irAE. 3 (6%) had serious AE (PG, adrenal insufficiency, liver disorder). |
| PDAC | PD-L1 | BMS-936559, CT00729664 | 1st or more | I | Dose escalation | Total, 207 PDAC, 16 | PDAC, 0 of 14 (0%) | MTD was not reached. irAE, 81 of 207 (39%), included rash, hypothyroidism, hepatitis, diabetes mellitus. |

*Ann Gastroenterol Surg.* 2018;2:289–303.

One matter is the **suppressive tumor microenvironments** including cytokines, immune cells, and immune checkpoint.
We found that the exhaustion markers (PD-1, TIM-3) are the critical matters for the efficacy, and that the **combination adjuvants of Poly-ICLC and LAG-3Ig** activates cancer specific immunity.
From **a phase I study of novel immunotherapy composed by the adjuvants and novel tumor antigens against various GI cancers, we observed the CTL induction in 16 of 17cases, the reduction of tumor markers in 10 of 17**.

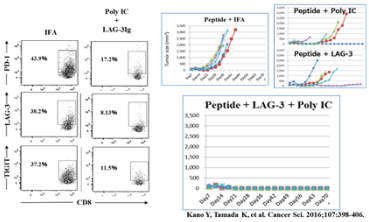
Concept of immunological status of various tumors or patients and implications for immunotherapy.




Dark tumor might need **immune checkpoint inhibitors** and agents to **resolve suppressive immunity**.

Patients with immune exhaustion might need additional treatment to **resolve the exhaustion**.

Novel Immune adjuvants; Poly(I:C) plus LAG-3-Ig




Kano Y, Tamada K, et al. Cancer Sci. 2016;107:398–406.

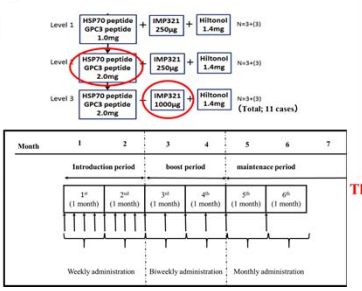
## YNP01 Study

**Outline**

- **First-in-Human study of peptide Vaccine in patient (HLA*A-24:02, 02:01, or 02:06) with advanced or relapsed Gastrointestinal Cancers**

**Investigational Drug**

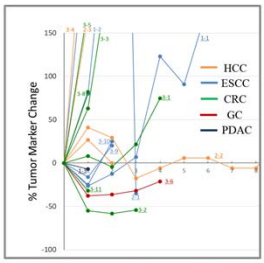
- HSP70 (Class I peptide) + GPC3 (Class I peptide) + Poly-ICLC (Hiltonol®) / Oncovir + LAG-3Ig (IMP321) / Immutep




**Endpoints**

- Safety
- Efficacy
  immunological, clinical

## Tumor markers, CTLs, and exhaustion markers




**The level of tumor markers was decreased in 10 patients out of 17 patients.**

| Level | Type | Site | OR | OS (m) | ELISPOT HSP70 | ELISPOT GPC3 |
|---|---|---|---|---|---|---|
| 1-1 | ESCC | Peritoneum | SD | 10.5 | 2+ | 2+ |
| 1-2 | ESCC | LN | PD | 2.4 | 4+ | 3+ |
| 1-3 | PDAC | Liver | PD | 2.8 | + | + |
| 2-1 | ESCC | LN | SD | 6.5 | 3+ | 3+ |
| 2-3 | HCC | Liver, Bone, LN | PD | 6.3 | + | 2+ |
| 2-2 | HCC | Lung | SD | 20.4 A | 3+ | 2+ |
| 3-1 | CRC | Liver | PD | 18.8 A | 5+ | 4+ |
| 3-2 | CRC | Lung, Pelvic | PD | 5.3 | 2+ | + |
| 3-3 | CRC | Liver, Lung | PD | 18.7 A | 3+ | 4+ |
| 3-4 | HCC | Lung | PD | 16.6 A | 3+ | 3+ |
| 3-5 | CRC | LN, Lung | PD | 9.6 | 4+ | 4+ |
| 3-6 | GC | Liver, LN | SD | 10.5 | 5+ | 5+ |
| 3-7 | HCC | Lung, Bone | SD | 11.9 A | 4+ | 4+ |
| 3-8 | CRC | Ling, Bone, Peri | PD | 10.8 | - | + |
| 3-9 | ESCC | Bone | PD | 2.3 | 5+ | 2+ |
| 3-10 | ESCC | LN | PD | 5.5 A | 3+ | 4+ |
| 3-11 | CRC | Lung, LN | PD | 4.7 A | 3+ | 2+ |

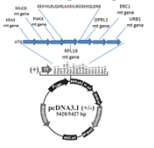
To enhance Neo-CTL, we have constructed **a unique binding prediction system** to HLA. We can narrow down to 20 mutations as candidate neoantigens by the prediction system.

List of nonsynonymous mutationa in adenocarcinoma of pancreas

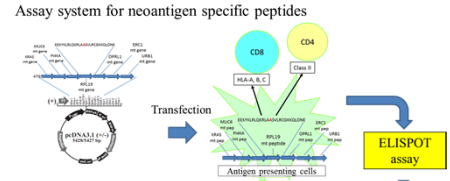
| CHROM | POS | ID_COSMIC | REF | ALT | NORMAL | TUMOR | Gene | amino_acid | FPKM_T | RNA_variant |
|---|---|---|---|---|---|---|---|---|---|---|
| chr12 | 25398284 | COSM49168,CO | C | A | 96:0:0:0 | 261:1:0:0 | KRAS | p.Gly12Val | 21.70 | PASS |
| chr22 | 21159405 | . | T | C | 46:1:0:0 | 109:2:0:0 | PHKA | p.Gln406Arg | 18.33 | PASS |
| chr9 | 21971111 | COSM1650884 | G | A | 62:1:0:0 | 135:1:0:0 | CDKN2A | p.Ha837yr | 9.20 | PASS |
| chr17 | 7578460 | COSM45883 | A | G | 45:0:0:0 | 461:0:0:0 | TP53 | p.Val157Ala | 9.10 | PASS |
| chr10 | 102265159 | COSM4208009 | G | T | 100:4:0:0 | 148:2:0:0 | SEC31B | p.Pro380Thr | 8.75 | PASS |
| chr21 | 33727868 | COSM3423907 | G | A | 53:0:0:0 | 131:1:0:0 | URB1 | p.Arg666Trp | 8.33 | PASS |
| chr18 | 48603023 | COSM4998306 | C | T | 46:0:0:0 | 71:0:0:0 | SMAD4 | p.Gln442* | 7.67 | PASS |
| chr6 | 32796793 | . | G | A | 22:0:0:0 | 28:0:0:0 | ITAP2 | p.Arg651Cys | 2.45 | PASS |
| chr17 | 37357494 | COSM4595243 | T | G | 100:1:0:0 | 128:0:0:0 | RPL19 | p.Ser12Ala | 945.75 | |
| chr6 | 31324025 | COSM4419059 | G | A | 202:5:0:0 | 360:4:0:0 | HLA-B | p.Arg180Trp | 124.60 | |
| chr2 | 238661994 | . | G | T | 91:0:0:0 | 149:0:0:0 | LRRFIP1 | p.Val176Phe | 46.95 | |
| chr16 | 85010001 | . | A | G | 34:1:0:0 | 140:0:0:0 | ZDHHC7 | p.Phe329Ser | 46.90 | |
| chr11 | 1017021 | . | T | A | 345:24:0:0 | 618:36:0:0 | MUC6 | p.His1927Leu | 41.24 | |
| chr6 | 136582401 | COSM5653193 | A | T | 18:1:0:0 | 160:0:0:0 | BCLAF1 | splice_donor_va | 31.37 | |
| chr4 | 190883077 | . | C | A | 112:0:0:0 | 246:0:0:0 | FRG1 | p.Leu244Ile | 26.57 | |
| chr14 | 105175978 | . | A | G | 71:0:0:0 | 189:1:0:0 | INF2 | p.Thr692Ala | 19.15 | |

Selection of target genes by AI


Vector encoding neoantigens




Assay system for neoantigen specific peptides




Candidate for neoantigen derived peptides

| No. | Gene Name | Mt. Sequence | Affinity |
|---|---|---|---|
| 1 | RPL19 | LRLQKRLAA | 4.1088 |
| 2 | RPL19 | RLQKRLAAS | 3.9688 |
| 3 | RPL19 | LQKRLAASV | 5.1873 |
| 4 | RPL19 | QKRLAASVL | 3.5681 |
| 5 | RPL19 | KRLAASVLR | 3.64 |
| 6 | RPL19 | RLAASVLRC | 3.7913 |
| 7 | RPL19 | LAASVLRCG | 3.9829 |
| 8 | RPL19 | AASVLRCGK | 3.4176 |
| 9 | RPL19 | ASVLRCGKK | 3.2378 |


**COI Disclosure Information**

**Lead Presenter/Responsible Researcher:**

**Shoichi Hazama**

**I have the following financial relationships to disclose.**

✓ **Grant/Research funding from:** **Toyo Kohan Corporation**
**NEC Corporation**
**CYTLIMIC Incorporation**

**Conclusion.** The combination therapy of immune-checkpoint inhibitors with YNP01 vaccine and neoantigens may be a promising strategy against gastrointestinal cancers.