***Supplemental Figures:***

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***Supplemental Figure 1. Gene-expression profiles of preclinical models yield a P. gingivalis infection-associated gene signature. A*,** Gene lists obtained from RNA sequencing of a *P. gingivalis*-infected cell line and naturally infected patients were overlapped to identify common upregulated and downregulated genes. The *p*-values were determined by hypergeometric tests. ***B.*** Expression of B7-H4 and KDM5B in a panel of 14 ESCC cell lines, as quantified by western blot. ***C,*** Human Kyse-150 cells were stimulated with *P. gingivalis* for 24 h, and the mRNA expression of a subset of 17 top-ranked immunity related genes and JmjC KDMs was measured by quantitative RT-PCR using total RNA. Data shown are the average fold changes of three independent experiments. Asterisks indicate statistically significant (*p*< 0.05) differences in changes in mRNA expression compared with control. ***D,*** Western blot of time-dependent B7-H4 and KDM5B levels following P. gingivalis exposure in YES-2 and Kyse-180 cells. GAPDH was used as an internal control. ***E,*** Supernatant generated from *P. gingivalis*-exposed explants-2 and -3 and *P. gingivalis*-exposed explants treated with CPI-455-neutralizing inhibitor were subjected to chemokine antibody array. Image represents the results of one of three independent experiments revealing similar patterns of expression.

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***Supplemental Figure 2. Quantification of the average signal (pixel density) and fold change of the pair of duplicate spots representing each chemokine.*** For each spot, the net optical-density level was determined by subtracting the background optical level (fibrinogen) from the total raw optical-density level. Each of the samples was analyzed on triplicate blots. *(A, DandF):* The average net optical intensity for each pair of chemokine spots is shown; *(B, CandE):* Fold change of Pg/control or Pg+455/Pg were calculated, control or Pg was set 1, respectively. Quantification of average signal and fold change of *explants 1, 2, 3* is shown in *Figure S2A-C, Figure S2D-E, Figure S2F-S3A,*respectively.

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***Supplemental Figure 3. Time course of chemokine expression of CXCL10, CXCL9, and CXCL11 in Kyse-150, -410 and -180 cells. A.*** Fold change ofPg+455/Pg for each pair of chemokine spots of *explants 3* is shown. Pg was set 1, respectively. ***B, C,*** We used total protein from ESCC cells in the presence or absence of P. gingivalis isolate and *P. gingivalis*-exposed cells in the presence of a JmjC KDM-neutralizing inhibitors (JIB-04; CPI-455) for further analyses at indicated time period. Chemokine expression was assessed by western blot, with GAPDH used as an internal control. In the current cell-culture model (Kyse-150, -410, and -180), background production of CXCL10, CXCL9, and CXCL11 was consistently detected in cells not infected with *P. gingivalis*. However, additional chemokine production was observed upon *P. gingivalis* infection (infected group) and showed slightly higher levels before rapidly declining (6–96 h). Furthermore, CXCL10, CXCL9, and CXCL11 were reproducibly detected at abundant levels in *P. gingivalis*-exposed cells that had been pre-incubated with the KDM5B-neutralizing inhibitors (CPI-455; JIB-04; rescued group). In this group, time-dependent chemokine production was maximal at 48 h. The response of the cells to infection became weaker in prolonged cultures,and chemokine production declined in some cases during the 96-h culture.

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***Supplemental Figure 4. KDM5B binds the promoters of CXCL10, CXCL11, and CXCL9 to alter their methylation status. A, B,*** Activated T cells were placed into the upper wells of a transwell system, and conditioned medium from tumor cells was loaded into the lower wells. T cell migration toward supernatantgenerated from sham-infected cells, *P. gingivalis*-exposed cells, and *P. gingivalis*-exposed cells treated CPI-455-neutralizing inhibitor in the presence of antibodies against *CXCL9, CXCL10*, and *CXCL11*, respectively, or cocktail antibodies concurrently targeting each chemokine. At 1-h post-incubation, the migrated T cells were assessed by flow cytometry. ***C,*** ChIP analysis of four regions of *CXCL10, CXCL11* and *CXCL9* for KDM5B binding. KDM5B specifically associated with regions close to sites of transcriptional initiation. ***D.*** *CXCL10, CXCL11,* and *CXCL9* expression in cells expressing wild-type (WT) or mutant (MT) KDM5B according to real-time RT-PCR. Expression of MT KDM5B caused reactivation of target genes.

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***Supplemental Figure 5. Immune responses to* P. gingivalis *infection in mice appear to be ineffective in resolving the infection.*** A, The numbers of *P. gingivalis* bacteria in esophageal biopsies from C57BL/6 mice over time. Mice (*n* = 70) that had been inoculated with a suspension of *P. gingivalis* containing 3 × 108 CFU of the bacteria were divided into seven groups (each containing 10 mice) that were sacrificed at different times. The numbers of CFU in homogenates of esophageal samples were determined as described in the Method. Each point represents the mean of two estimations for a single mouse. Horizontal bars represent the geometric means for mice (*n* = 10) sacrificed at each time point. B1, The inflammation in esophageal biopsies from C57BL/6 mice with P. gingivalis infection over time. The extent of histological inflammation was assessed at indicated times after infection by evaluating H&E-stained sections as described in Materials and Methods. Each point represents the mean of two estimations for a single mouse. Horizontal bars represent the geometric means for mice (*n* = 10) sacrificed at each time point. B2, Anti-*P. gingivalis* antibodies in the sera of C57BL/6 mice infected by *P. gingivalis*. Specific IgG and IgA class antibodies in the sera of *P. gingivalis*-infected mice were detected by ELISA. The results are presented as *A*405–492 readings which correspond to the values given by diluted serum samples (1:100). Each point represents the mean of triplicate determinations for a single mouse. Horizontal bars represent the geometric means for mice (*n* = 10) sacrificed at each time point. B3, Anti-*P. gingivalis* antibodies in the esophageal contents of C57BL/6 mice infected by *P. gingivalis*. Specific IgG and IgA class antibodies in the esophageal contents of *P. gingivalis*-infected mice were detected by ELISA. The results are presented as described for Fig. B2, except that esophageal content samples were diluted 1:10. Each point represents the mean of triplicate determinations for a single mouse. Horizontal bars represent the geometric means for mice (*n* = 10) sacrificed at each time point. Differences in the bacterial loads, inflammation or antibody responses between groups of animals were determined by the Wilcoxon rank sum test. Differences were considered significant for a P of < 0.05. C, Memory response against P. gingivalis infection in mice re-challenged with higher bacterial load. Following re-challenge, mice dosed according to the schema were evaluated for colonization by *in situ* hybridization at the indicated time points. Detection of *P. gingivalis* mRNA in esophageal mucosa. Positive staining is diffusely present in the cytoplasm as a punctate or granular appearance. Positive signals were detected in mucosa and submucosa.

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***Supplemental Figure 6. Phased administration of CPI-455 and the anti-B7-H4 antibody improves anti-tumor efficacy and induces complete tumor regressions in a humanized mice model. A,*** Xenografts were treated with CPI-455 (50 mg/kg, daily, intraperitoneally) and anti-B7-H4 Ab (188) (500 μg/mouse, weekly, intraperitoneally), followed by further administration using sequential (CPI-455 and anti-B7-H4 antibody initiated on days 6 and 20, respectively) or phased (CPI-455 and anti-B7-H4 antibody initiated on days 6 and 13, respectively) dosing schedules. Experimental schema is presented. ***B subpanel a-d,*** Individual tumor-growth curves (blue lines) are shown overlaid on top of those from a control (black lines) untreated tumor. CR rates at study termination are shown. ***C*,** Patient-derivedxenografts were treated as indicated schema following CPI-455 (50 mg/kg) and CPI-455 (75 mg/kg) treatments. Phased combined treatment for 14 days with CPI-455 starting on day 6 and combined starting 1 week later with anti-B7-H4 on day 13 for 3 weeks or extended phased combined treatment involving a 28-day administration of CPI-455 is shown. ***D,*** Individual tumor-growth curves are shown for the anti-B7-H4 antibody (188) (500 μg/mouse, weekly, intraperitoneally) and overlaid on top of those from a control untreated tumor. CR rates at study termination are shown.

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***Supplemental Figure 7. Adoptive transfer of human T lymphocytes causes tumor remission in NSG mice that is exacerbated by Anti-B7-H4 Ab and CPI-455 and abrogated by CD8+ T lymphocytes depletion. A,*** Mice bearing human ESCC tumors but without T cell transfer did not show any improvement upon combined treatment when compared with the control or monotherapy group. Thus, depletion of immunity significantly abrogate the antitumor effect of the combinatorial treatment (*upper images*). ***B,*** Results depict mean ± SEM of tumor growth curves (*n*= 6 mice per group; *lower chart*). Experiments were repeated three times, rendering similar results.

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### *Supplemental Figure 8. Concurrent expression of B7-H4 and KDM5B correlates with less-dense infiltrates of CD8+ T lymphocytes and more severe* P. gingivalis *infection* *A.* Significant positive correlation between P. gingivalis load and expression of KDM5B, B7-H4, and two molecules on ESCCs. *B.* Significant negative correlation between CD8 and KDM5B, B7-H4, and two molecules on ESCC subjects. The Spearman correlation test was used to determine statistical significance.

Supplementary Table 1: Primers for q-RT-PCR analysis

|  |  |  |
| --- | --- | --- |
| Gene | Sequence (5′-3′) | Sequence (5′-3′) |
| CXCL9 | GAGCAGTGTGGAGTTCGAGG | TCCGGATCTAGGCAGGTTTG |
| CXCL10 | AATGAGGGCCATAGGGAAGC | AGCCATCCACTGGGTAAAGG |
| CXCL11 | GAGACCCTGGCTAGAGATGC | CATGGCACAGCGAAAGTTGG |
| B7-H4 | GGCACAGCTCAACCTCATCT | TGATCTTTCTCCAGCACACG |

Supplementary Table 2: Primers for ChIP analysis and qRT-PCR

|  |  |  |
| --- | --- | --- |
| Gene | Sequence (5′-3′) | Sequence (5′-3′) |
| CXCL9 | AGGGTTTCCCCCAGCACAATCA | ACCAGCAAGATGATGCCCAAGAGG |
| CXCL10 | GGAATGTCTCAGAAAACGTGGGGC | ACCTTCGAGTCTGCAACATGGGA |
| CXCL11 | GCGGGAGTAGCAGGTAATCT | CAGTTGGCAGCTCCTTTTCT |
| CCL2 | GCTCACCCAGGCACAAAG | GCTGTGTGCAGCAGAAGG |
| CCL5 | GGGTTGGGAGGGAAGGAAAG | CCCATCCATCTGAGAGCGAC |

**Supplemental Table 3. Locations of controls and capture antibodies in the chemokine array.**

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**Supplemental Table 4. Kaplan–Meier analysis of survival after treatment with CPI-455 and/or the anti-B7-H4 antibody in a humanized mice model.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** |  |  | **Log rank *p*** | | |
| **Median**  **Survival**  **(days)** | **Complete**  **Responders** | **vs.**  **Ctrl** | **vs. Anti-B7H4**  **Antibody**  **monotherapy** | **vs. CPI-455**  **monotherapy** |
| **Ctrl** | **20** | **0** |  | **\_** | **\_** |
| **Anti-B7-H4 monotherapy** | **21** | **0** | **0.876** | **\_** | **\_** |
| **CPI-455**  **monotherapy** | **26** | **0** | **0.071** | **\_** | **\_** |
| **Sequential**  **combination**  **schedule** | **27** | **0** | **0.006** | **0.021** | **0.213** |
| **Phased**  **combination**  **schedule** | **35** | **2** | **<0.001** | **<0.001** | **0.006** |

**Supplemental Table 5. Kaplan–Meier analysis of survival for phased versus extended phased combined treatment with CPI-455 and/or the anti-B7-H4 antibody in a humanized mouse model.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Median survival**  **(days)** | **CR** | **Rejection**  **Secondary**  **tumors** | **Log rank *p*** | | |
| **vs. Ctrl** | **vs. Anti-B7-H4 Antibody**  **monotherapy** | **vs. corresponding**  **CPI-455**  **dose monotherapy**  **group** |
| **Ctrl** | **21** | **0** | **\_** |  |
| **Anti-B7-H4 monotherapy** | **22** | **0** | **\_** | **0.426** |
| **50 mg/kg CPI-455**  **monotherapy** | **28** | **0** | **\_** | **0.154** |
| **75 mg/kg CPI-455 monotherapy** | **31** | **1** | **\_** | **0.012** |
| **50 mg/kg CPI-455+ anti-B7-H4 Phased Combination** | **38** | **2** | **2/2** | **0.001** | **0.005** | **0.010** |
| **50 mg/kg CPI-455+ anti-B7-H4 Extended Phased Combination** | **50** | **3** | **3/3** | **<0.001** | **<0.001** | **<0.001** |
| **75 mg/kg CPI-455+ anti-B7-H4 Phased Combination** | **44** | **3** | **3/3** | **0.001** | **0.001** | **0.298** |
| **75 mg/kg CPI-455+ anti-B7-H4 Extended Phased Combination** | **63** | **5** | **5/5** | **<0.001** | **0.001** | **0.080** |

**Supplemental Table 6. Relationship between B7-H4 and KDM5B expression, P. gingivalis load, and CD8+ T cell count in ESCC tissues according to IHC.**

