

# Investigating the tumor microenvironment using QIAGEN Ingenuity

## Pathway Analysis (IPA) (II)

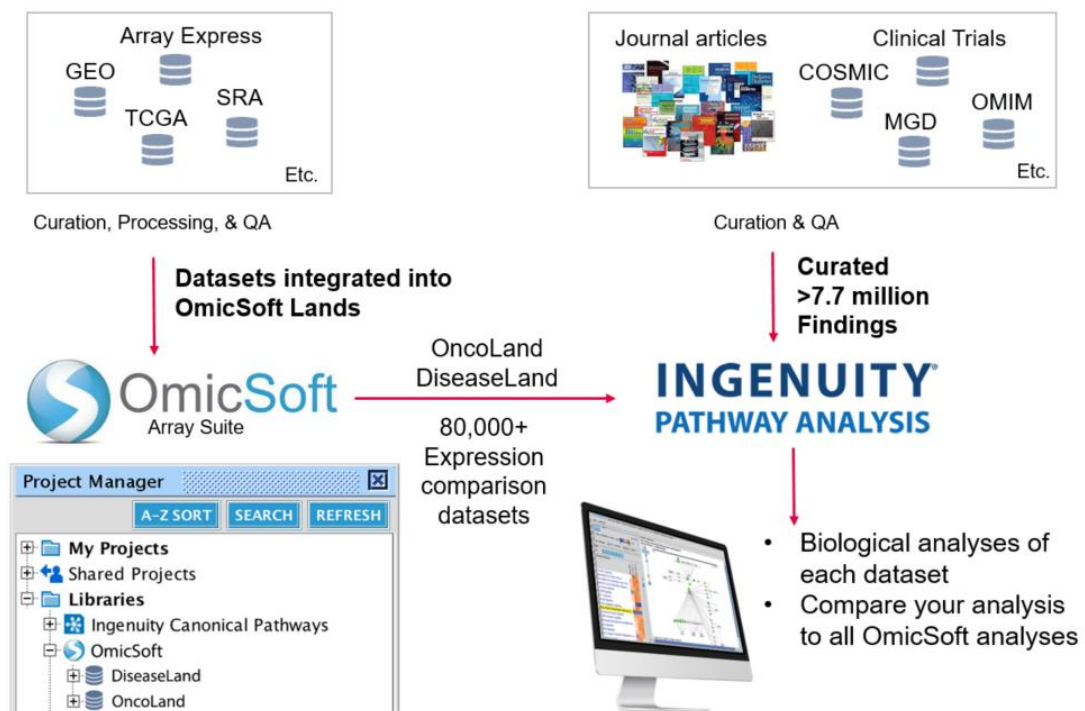
### 如何利用 QIAGEN IPA 來研究腫瘤的微環境 (下)

本次的 IPA Case Study，一樣會接續應用 IPA 知識庫所累積及整合的資料來源，輕鬆的幫助您了解研究中關鍵的目標分子和疾病適應症關係，將延續上次的範例，教大家如何套用真實數據做分析比較，能幫助大家更深入系統生物學的應用。

- 建立目標分子連接到 tumor microenvironment (TME) 和疾病的網絡
- 剖析癌症中的關鍵免疫分子
- 分析 TME 途徑內表達變化的影響
- 建立與免疫反應有關的監管概況

## QIAGEN Knowledge Base and OmicSoft Lands

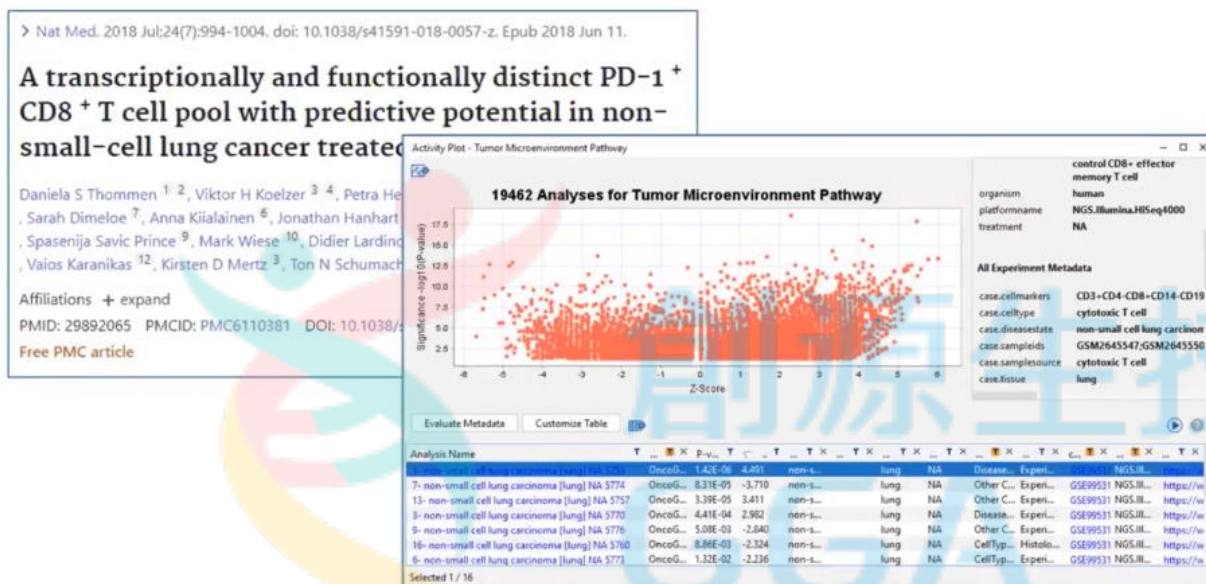
QIAGEN 知識庫目前經過專業人員校正及跟自動化比對的資料，及整合了 OmicSoft 中八萬筆以上的疾病、致癌知表現資料，也有第三方資料庫如 COSMIC(癌症)、Target Scan(miRNA)、OMIM 等，周周更新累積 20 年有 7.7 百萬資料，也因為有這麼龐大的資料庫，使用戶們可以很好的利用 IPA 找尋出來關鍵的分子的關係。



# Case Study: Investigating the tumor microenvironment

THOMMEN, Daniela S., et al. A transcriptionally and functionally distinct PD-1+ CD8+ T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade. *Nature medicine*, 2018, 24.7: 994-1004.

Interrogate immunological mechanisms and the TME with internal or public data

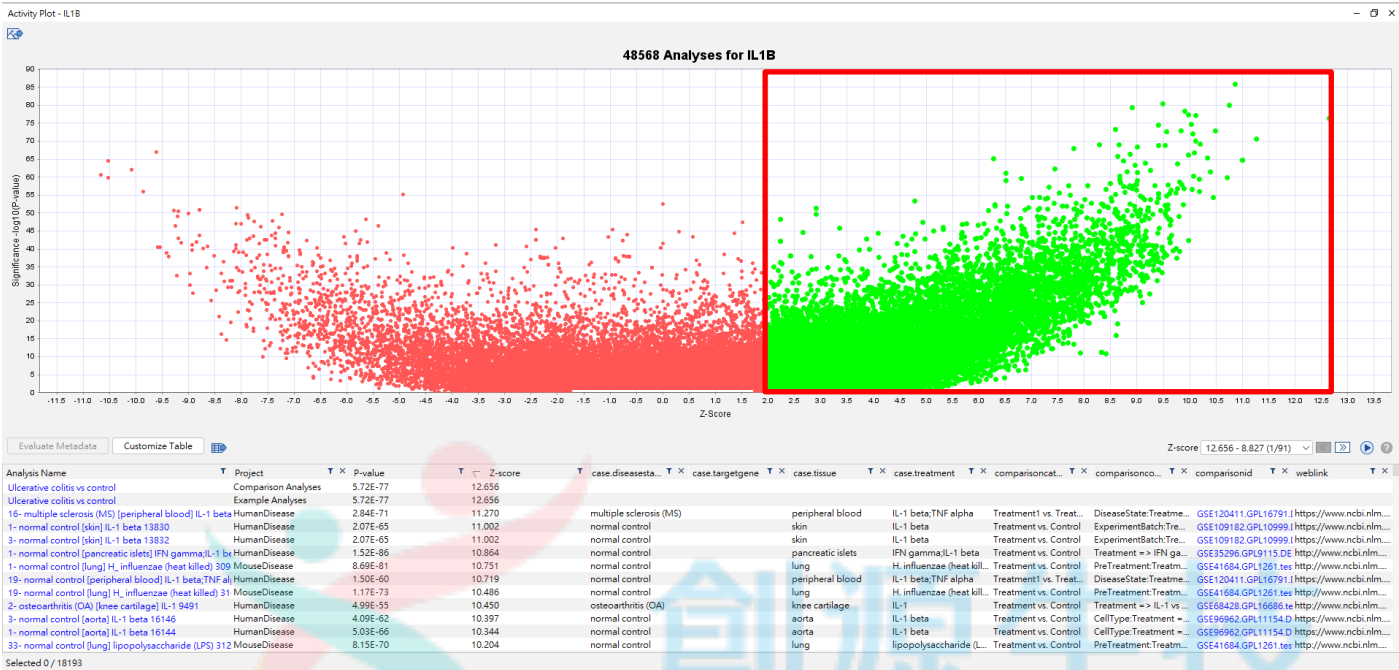


上次案例我們分享了如何找出 IL1B, Non-small cell lung carcinoma(LC) & Tumor Microenvironment Pathway (TMP)之間的關聯性，而這次的案例將分享如何透過 QIAGEN 知識庫及公開資料來探討免疫機制及腫瘤微環境(TME)之關係，並比較各個不同非小細胞癌的病例之間的表现变化及分析。

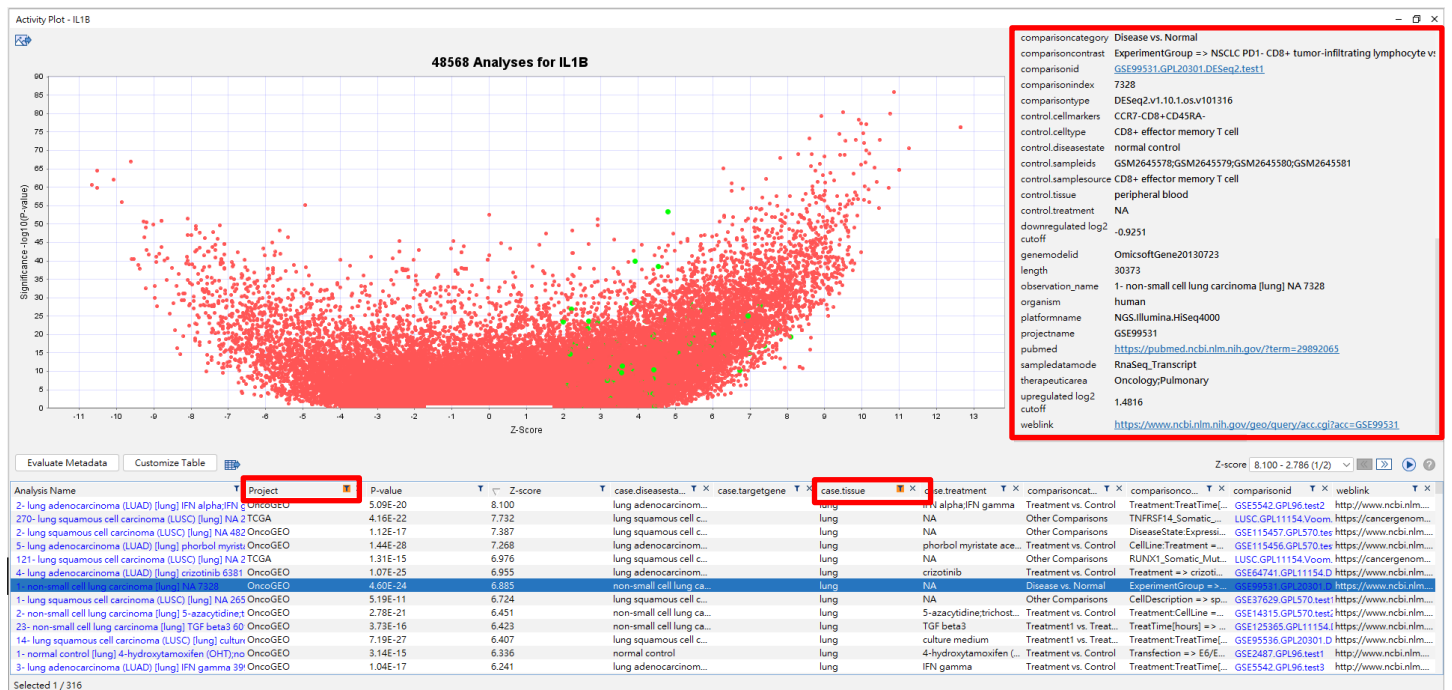
## 資料建立 (Search – Activity Plot)

The screenshot shows the QIAGEN IPA software interface. The search bar contains "IL1B". The search results show one item: IL1B, with synonyms including IL-1, IL1-BETA, IL-1F2, IL-1 β, interleukin 1 beta, Interleukin 1 β, OAF, Osteoclast-Activating Factor, Pro-IL-1beta, and Pro-IL-1β. The "Activity Plot" button is highlighted with a red box. A dialog box titled "Select Entity Type" is open, showing "Upstream Regulator" selected in the "Entity Type" dropdown menu.

首先在 IPA 的軟體介面，基因欄位搜尋"IL1B"，目標物勾選後，點選 Activity Plot，Type 選擇 Upstream Regulator.



搜尋後的結果，可得到 IL1B 約五萬篇分析資料有表現資料，我們可以先將 Z-Score 大於 2 的資料集框起來(上圖綠色處)，可得到各筆資料之名稱、計畫、P-value、Z-score、組織、疾病狀態...等相關資訊。



進一步，我們針對 Project: OncoLand 及 case.tissue: \*lung\* 做資料篩選，可得到約 300 筆相關之分析資料，點選任一個有興趣的 analysis name，右上方會出現此筆資料之數據注釋(metadata)，包含了細胞型態、疾病狀況、組織、疾病及正常態的比較資訊，以及所有實驗資料之細節。(metadata之最下方為 weblink，可自動連結至 NCBI 的網站，查找有興趣的文獻實驗，如下圖。)

COVID-19 is an emerging, rapidly evolving situation.  
 Get the latest public health information from CDC: <https://www.coronavirus.gov>.  
 Get the latest research from NIH: <https://www.nih.gov/coronavirus>.  
 Find NCBI SARS-CoV-2 literature, sequence, and clinical content: <https://www.ncbi.nlm.nih.gov/sars-cov-2/>.

NCBI > GEO > Accession Display

Scope: Self Format: HTML Amount: Quick GEO accession: GSE99531

**Series GSE99531** Query DataSets for GSE99531

Status Public on May 22, 2018  
 Title A transcriptionally und functionally distinct PD-1+ CD8+ T cell pool with predictive potential in non-small cell lung cancer treated with PD-1 blockade  
 Organism *Homo sapiens*  
 Experiment type Expression profiling by high throughput sequencing  
 Summary Evidence from mouse chronic viral infection models suggests that CD8+ T cell subsets characterized by distinct expression levels of the receptor PD-1 diverge in their state of exhaustion and potential for reinvigoration by PD-1 blockade. However, it remains unknown whether T cells in human cancer adopt a similar spectrum of exhausted states based on PD-1 expression levels. We compared transcriptional, metabolic, and functional signatures of intratumoral CD8+ T lymphocyte populations with high (PD-1T), intermediate (PD-1N) and no PD-1 expression (PD-1-) from non-small cell lung cancer patients. We observed that, PD-1T T cells show a markedly different transcriptional and metabolic profile as compared to PD-1N and PD-1- lymphocytes, as well as an intrinsically high capacity for tumor recognition. Furthermore, while PD-1T lymphocytes are impaired in classical effector cytokine production, they produce CXCL13 that mediates immune cell recruitment to tertiary lymphoid structures. Strikingly, the presence of PD-1T cells was strongly predictive for both response and survival in a small cohort of non-small cell lung cancer patients treated with PD-1 blockade. The characterization of a distinct state of tumor-reactive, PD-1 bright lymphocytes in human cancer, which only partially resembles that seen in chronic infection, provides novel potential avenues for therapeutic intervention.

Overall design Intratumoral CD8+ T cells from 11 non-small cell lung cancer patients that were sub-sorted into PD-1-high (PD-1T), PD-1-intermediate (PD-1N) and PD-1-negative (PD-1-) cells, were sequenced using Illumina HiSeq4000. In addition, peripheral blood effector memory T cells from 4 healthy donors were sequenced using Illumina HiSeq4000.

接著我們將帶大家分析 TME 途徑內表達變化的影響，先點選 1-non-small cell lung carcinoma[lung] NA7328 的 IPA 分析資料，可得到已透過 IPA 核心分析的 GSE99531 資訊，包含圖形、路徑、上游調控因子、疾病功能、調控、分子網路及資訊比對。

Expression Analysis - 1-non-small cell lung carcinoma [lung] NA 7328

Summary Graphical Summary **Canonical Pathways** Upstream Analysis Diseases & Functions Regulator Effects Networks Lists My Pathways Molecules Analysis Match

Chart Overlapping

Customize Chart  Horizontal  Vertical Overlay:  Stacked Bar Chart

Systemic Lupus Erythematosus In B Cell Signaling Pathway  
 Hepatic Fibrosis / Hepatic Stellate Cell Activation  
 Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses  
**Tumor Microenvironment Pathway**  
 Dendritic Cell Maturation  
 HMGB1 Signaling  
 Communication between Innate and Adaptive Immune Cells  
 Type 1 Diabetes Mellitus Signaling

1-non-small cell lung carcinoma [lung] NA 7328  
 Tumor Microenvironment Pathway  
 molecules=24  
 z-score=4.491 (biased)  
 -log(p-value)=5.839  
 p-value=1.45E-06  
 ratio=24/144 (0.167)

24 molecule(s) associated with Tumor Microenvironment Pathway at 1-non-small cell lung carcinoma [lung] NA 7328 [Ratio: 24/144 (0.167)] [z-score: 4.491] [p-value: 1.45E-06]

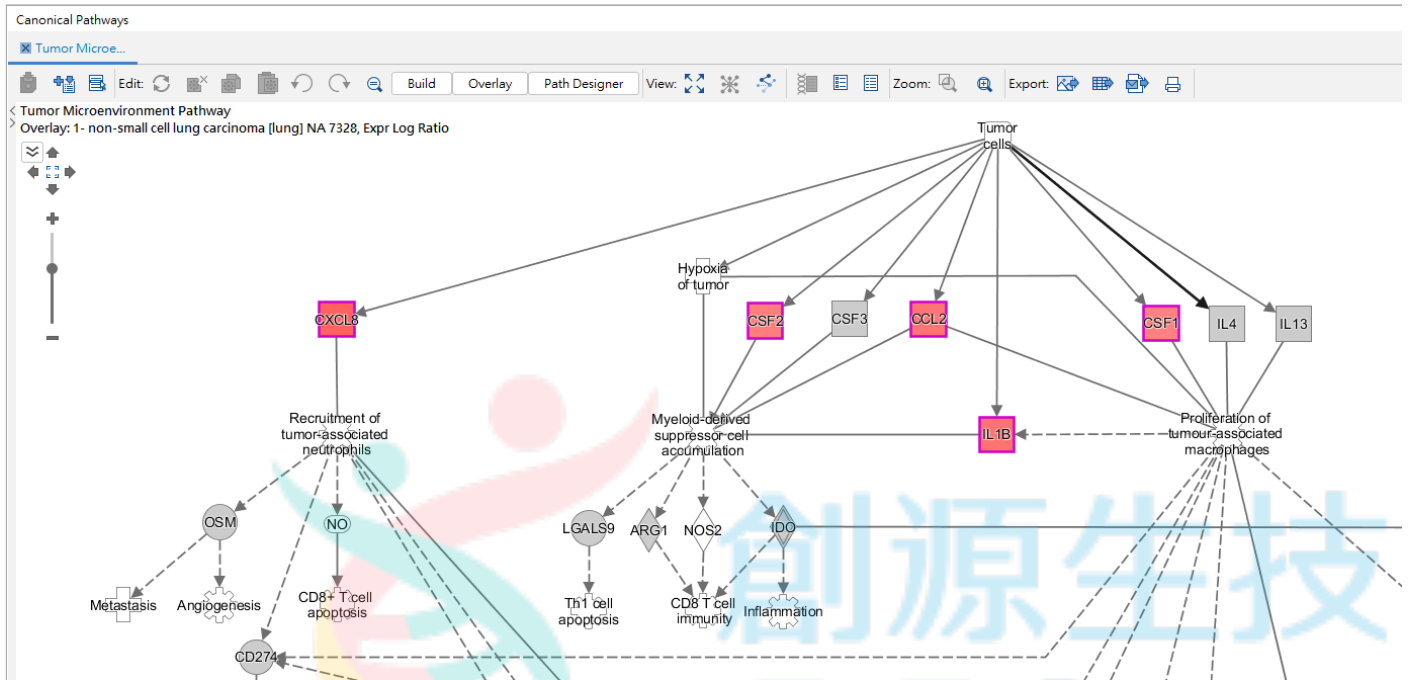
Add To My Pathway Add To My List Create Dataset Customize Table Expand

| Symbol | Entrez Gene Name                              | Identifier | Measurement | Expr Log Ratio | Expr p-value | Expr p-value | Expr Intensity/RPK... | Expr Intensity/RPK... | Expr Other | Expected | Location            |
|--------|---|------------|-------------|----------------|--------------|--------------|-----------------------|-----------------------|------------|----------|---------------------|
| CCL2   | C-C motif chemokine ligand 2                  | CCL2       | ↑1.665      | 8.00E-04       | 6.11E-03     | 280.411      | 1.472                 | ↑1.000                | ↑          | Up       | Extracellular Space |
| CSF1   | colony stimulating factor 1                   | CSF1       | ↑1.516      | 1.08E-03       | 7.75E-03     | 4738.295     | 166.672               | ↑1.000                | ↑          | Up       | Extracellular Space |
| CSF2   | colony stimulating factor 2                   | CSF2       | ↑1.562      | 6.12E-04       | 4.88E-03     | 89.574       | 6.392                 | ↑1.000                | ↑          | Up       | Extracellular Space |
| CXCL8  | C-X-C motif chemokine 8                       | IL8        | ↑1.907      | 9.94E-05       | 1.07E-03     | 3443.022     | 3.205                 | ↑1.000                | ↑          | Up       | Extracellular Space |
| FASLG  | Fas ligand                                    | FASLG      | ↑2.340      | 5.19E-16       | 7.02E-14     | 3168.064     | 446.204               | ↑1.000                | ↑          | Up       | Extracellular Space |
| FN1    | fibronectin 1                                 | FN1        | ↑1.589      | 1.44E-03       | 9.69E-03     | 1540.467     | 22.534                | ↑1.000                | ↑          | Up       | Extracellular Space |
| FOS    | Fos proto-oncogene, AP-1 transcription factor | FOS        | ↑1.860      | 1.32E-07       | 3.52E-06     | 38371.630    | 8013.929              | ↑1.000                | ↑          | Up       | Nucleus             |
| FOXO4  | forkhead box O4                               | FOXO4      | ↑1.860      | 3.60E-08       | 1.14E-06     | 427.451      | 86.408                | ↑1.000                | ↓          | Down     | Nucleus             |

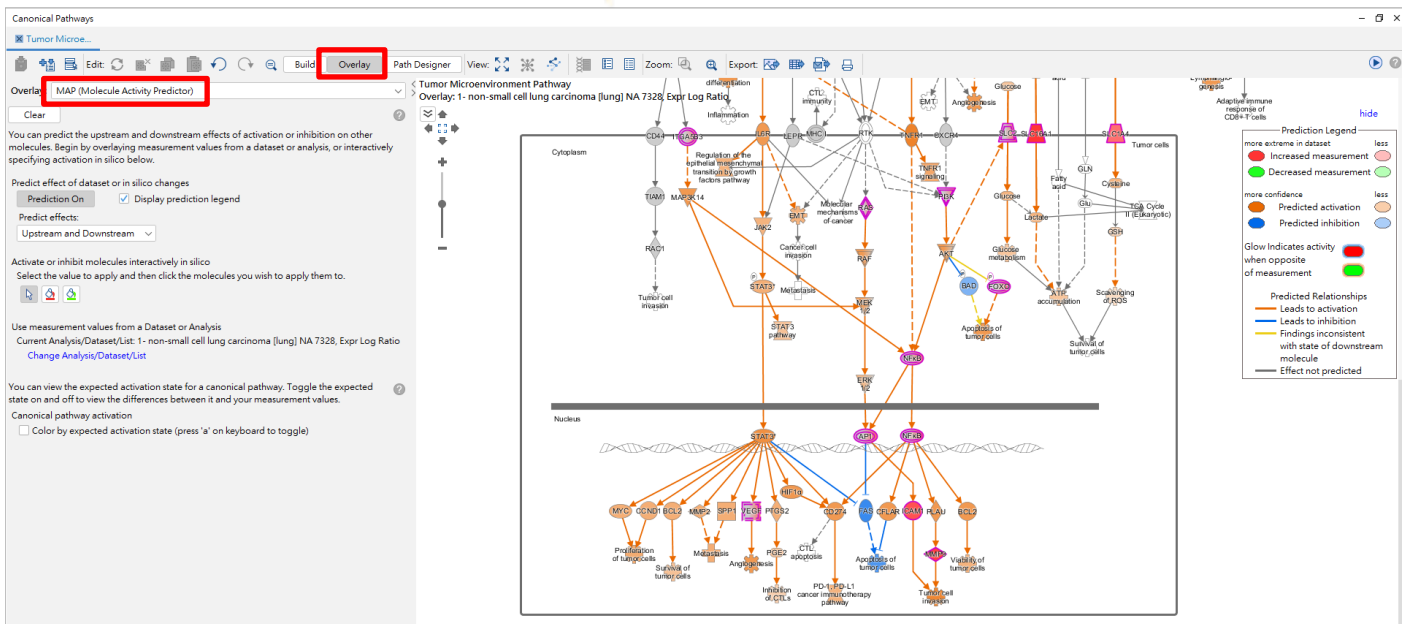
點選"Canonical Pathways"，可得到 IPA 找出所有相關(p-value)及影響(z-score)之生物路徑，選擇我們今天的主題 TME pathway，在 NA 7328 中共有 24 個分子參與，Z-score: 4.491 預測此路徑為活化表現。



下方的詳細資料則會顯示完整 24 個分子的資訊，包含名稱、實驗數據、預測表現、位置。



點開 TME 路徑圖，圖中有顏色的分子表示此份文獻有影響的，紅色的為高表現，綠色的為低表現，我們可以發現上游的 Tumor cells 可影響到 CXCL8、CSF1、CSF2、CCL2、IL1B 分子，進而導致(leads to)與腫瘤反應、增殖(recruitment、proliferation)等功能。



另外我們也可以使用功能(overlay -> MAP)，透過 IPA 快速預測在 TMP 下游腫瘤細胞的影響，可看到於細胞質活化 SLC1A4、SCLC16A1、PI3K、NFkB 等分子，進而活化了細胞核中 STAT3、MYC、BCL2... 等分子，並增加了腫瘤細胞的 proliferation、survival 及 apoptosis 相關表現及 PD-1, PD-L1 cancer immunotherapy pathway.

The screenshot shows the Omicsoft software interface. In the top right, a search bar contains "GSE99531". Below it, search results are displayed, including "16- non-small cell lung carcinoma [lung] NA 7335". In the bottom right, a table titled "Case/Control Differences" shows key differences between the two groups. A red box highlights the "Add more..." button in the matching molecules section.

| Key         | Case                                  | Control                    |
|-------------|---------------------------------------|----------------------------|
| cellmarkers | CD3+CD4-CD8+CD14-CD19-CD45+CD56-PD1hi | CD3+CD4-CD8+CD14-CD19-CD45 |

接著我們運用其他組肺癌的實驗資料，來比較 TMP 路徑中分子的變化，在 Overlay -> Analyses, Datasets & Lists -> 選擇 Add more，於搜尋列填上 "GSE99531" 後，可得到 24 組肺癌實驗數據及分析數據，這邊先選擇組別 16 後，點選 Overlay Now 以進行比較。

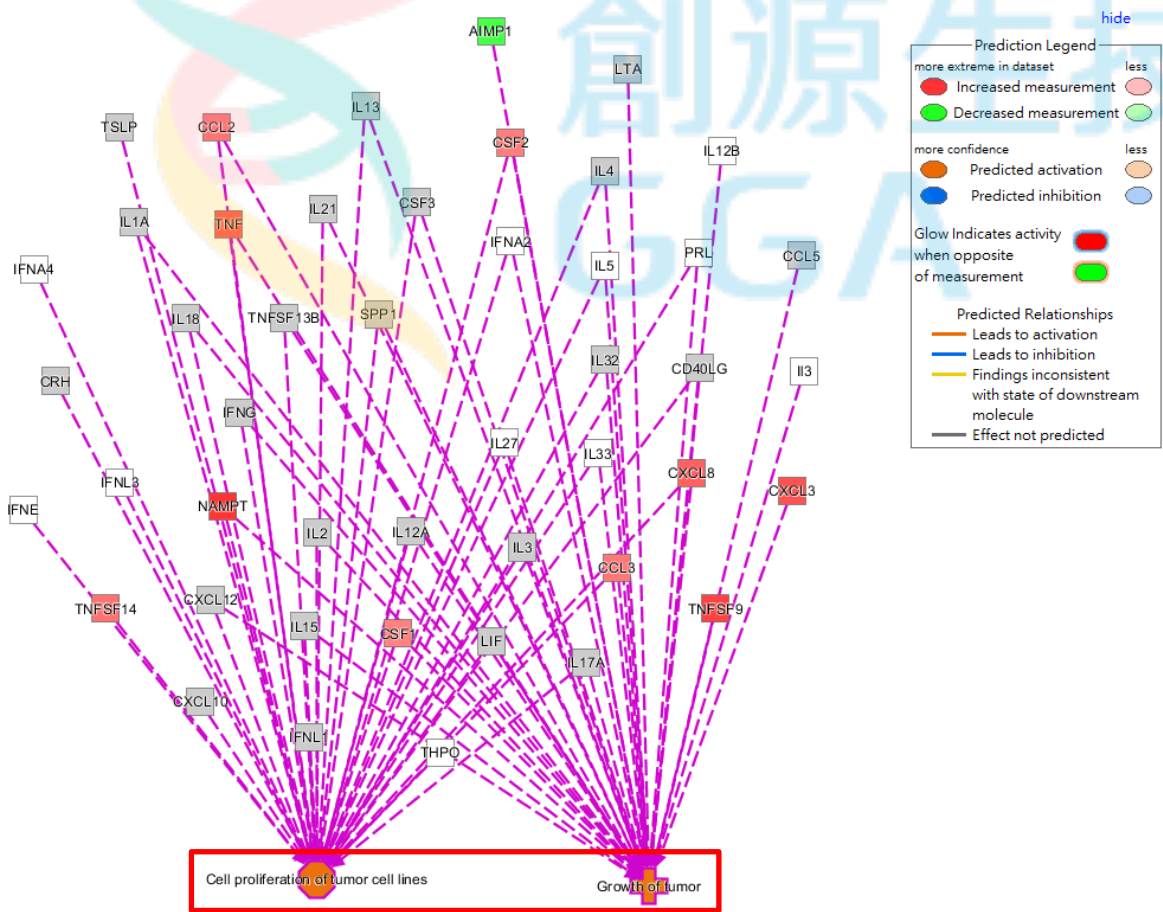
The screenshot shows the Omicsoft software interface with a pathway diagram and a list of matching molecules. The pathway diagram, titled "Tumor Microenvironment Pathway", shows various signaling molecules and their interactions. The list of matching molecules includes AP1, CCL2, CSF1, CSF2, CTLA4, CXCL8, and FASLG. A red box highlights the AP1 molecule in the list, showing its expression log ratio for group 16 as -1.935.

| Symbol | Display name | Index | Measurement                                      |
|--------|--------------|-------|--|
| AP1    | AP1          | 1     | 16- non-small cell lung carcinoma [lung] NA 7335 |
| CCL2   | CCL2         |       |  |
| CSF1   | CSF1         |       |  |
| CSF2   | CSF2         |       |  |
| CTLA4  | CTLA4        |       |  |
| CXCL8  | CXCL8        |       |  |
| FASLG  | FASLG        |       |  |

Overlay 後可進一步比較兩組 PD1 於肺癌中表現不同的實驗資料，Ap1 於組別 1 之 Log Ratio 為 1.86，於組別 16 為 -1.935，不同的活性表性，也進而影響了下游功能不同的表現。

| Upstream Regulator | Expr Log Ratio | Molecule Type | Predicted Activation State | Activation z-score | Flags | p-value of overlap | Target Molecules in Dataset           | Mechanistic Network |
|--------------------|----------------|---------------|----------------------------|--------------------|-------|--------------------|---------------------------------------|---------------------|
| IL17C              | +0.573         | cytokine      | Activated                  | 2.205              | bias  | 6.53E-03           | ↑CXCL2, ↑CXCL3, ↑IL18, ↑IL6, ...all 5 | 267 (8)             |
| IL33               |                | cytokine      | Activated                  | 5.179              | bias  | 1.01E-15           | ↑ABCG1, ↑APOE, ↑AREG, ...all 49       | 325 (15)            |
| IL26               | +0.395         | cytokine      | Activated                  | 2.201              | bias  | 2.06E-07           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 6      | 316 (18)            |
| TSLP               | +0.721         | cytokine      | Activated                  | 2.408              | bias  | 1.09E-04           | ↑CCR7, ↑CXCL3, ↑CXCL4, ...all 8       | 224 (17)            |
| THPO               | +1.907         | cytokine      | Activated                  | 3.991              | bias  | 7.81E-07           | ↑AURKA, ↑BIRC3, ↑CD83, ...all 17      | 232 (10)            |
| CXCL5              | +1.516         | cytokine      | Activated                  | 2.980              | bias  | 5.93E-06           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 14     | 331 (24)            |
| CSF1               | +1.516         | cytokine      | Activated                  | 5.241              | bias  | 1.44E-16           | ↑ADGRE5, ↑APOE, ↑CXCL3, ...all 49     | 351 (17)            |
| TNFSF14            | +1.700         | cytokine      | Activated                  | 3.284              | bias  | 9.34E-08           | ↑BIRC3, ↑CXCL2, ↑CXCL3, ...all 11     | 287 (16)            |
| PRL                |                | cytokine      | Activated                  | 3.820              | bias  | 1.55E-06           | ↑BIRC3, ↑CXCL2, ↑CXCL3, ...all 33     | 316 (18)            |
| TNFSF12            | +0.595         | cytokine      | Activated                  | 3.967              | bias  | 2.76E-07           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 17     | 254 (18)            |
| CCL3               | +1.526         | cytokine      | Activated                  | 2.417              | bias  | 1.03E-07           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 10     | 216 (16)            |
| NAMPT              | +2.593         | cytokine      | Activated                  | 2.588              | bias  | 2.70E-06           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 11     | 302 (16)            |
| IL27               |                | cytokine      | Activated                  | 3.758              | bias  | 2.54E-15           | ↑AHR, ↑CXCL2, ↑CXCL3, ...all 34       | 330 (16)            |
| AIMP1              | +1.082         | cytokine      | Activated                  | 2.777              | bias  | 3.87E-08           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 8      | 276 (21)            |
| IL5                |                | cytokine      | Activated                  | 4.546              | bias  | 2.21E-14           | ↑AREG, ↑CXCL3, ↑CXCL4, ...all 49      | 331 (16)            |
| CXCL10             | +1.222         | cytokine      | Activated                  | 2.567              | bias  | 2.03E-05           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 7      | 291 (21)            |
| IL2                |                | cytokine      | Activated                  | 2.399              | bias  | 3.32E-05           | ↑ANPEP, ↑CXCL4, ↑CXCL5, ...all 13     | 319 (19)            |
| IL3                | +1.003         | cytokine      | Activated                  | 4.364              | bias  | 8.66E-08           | ↑ADGRE5, ↑AREG, ↑BIRC3, ...all 40     | 260 (19)            |
| IL4                | +0.557         | cytokine      | Activated                  | 3.639              | bias  | 9.35E-17           | ↑ACSL5, ↑AHR, ↑AHLA1, ...all 107      | 364 (15)            |
| SPP1               | +1.102         | cytokine      | Activated                  | 2.048              | bias  | 1.45E-10           | ↑AURKA, ↑CXCL3, ↑CXCL4, ...all 31     | 350 (20)            |
| CCL2               | +1.665         | cytokine      | Activated                  | 2.230              | bias  | 4.24E-07           | ↑AREG, ↑CXCL3, ↑CXCL4, ...all 17      | 295 (21)            |
| LTA                | +0.122         | cytokine      | Activated                  | 2.813              | bias  | 2.45E-04           | ↑CXCL2, ↑CXCL3, ↑CXCL4, ...all 9      | 311 (19)            |
| CSF3               | +0.048         | cytokine      | Activated                  | 3.508              | bias  | 3.37E-16           | ↑BIRC3, ↑CXCL3, ↑CXCL4, ...all 40     | 330 (18)            |
| IFNL1              | +0.012         | cytokine      | Activated                  | 3.053              | bias  | 3.67E-03           | ↑ATF3, ↑CXCL2, ↑CXCL3, ...all 11      | 352 (15)            |
| IL21               | +0.103         | cytokine      | Activated                  | 3.495              | bias  | 1.42E-14           | ↑ARG2, ↑CXCL2, ↑CXCL3, ...all 35      | 338 (15)            |
| CXCL12             | +0.356         | cytokine      | Activated                  | 3.265              | bias  | 1.15E-06           | ↑AIF1, ↑BIRC3, ↑CXCL2, ...all 27      | 232 (17)            |

最後我們可以在回去 IPA 分析的頁面，觀看組別 1 的 Upstream Analysis 的分析，先篩選分子型態”Cytokine”，並將 Z-score 做排序，可得到 71 個活化非小細胞癌的上游調控因子，去探討文獻資料及實驗資料預測後，與腫瘤功能相關的調控機制。



上圖即為統整與 Cytokine 相關之上游調控因子，並加入腫瘤細胞增生(Cell proliferation of tumor cell lines)、腫瘤生長(Growth of tumor)功能之分子交互作用網路，可藉由實驗數據及 IPA 自動預測功能，快速得到分子與功能間上下游的關係，協助我們了解三者間的調控機制。

總結一下此次的範例介紹包含了基礎及進階功能的應用，我們可先透過 IPA 的資料庫建立，先探討有興趣的分子、疾病及路徑，並利用”connect”、”Path Explore”、”MAP”等功能串接 IL1B, LC & TMP 三者間關鍵的交互作用。

- 如何利用 IPA 資料庫搜尋，快速搜尋各分子之資料，及兩兩之間關係，並快速整理現有文獻資訊。
- 利用關鍵目標分子建立交互作用網路，並透過計算預測方式了解各分子間，是如何去影響網路內的成員。

再使用”Activity Plot”、”Core Analysis”、”Canonical Pathway”、”Upstream Analysis”等功能串接 IL1B, LC & TMP 三者，與實驗數據比對、路徑比較及上游調控預測。

- 使用真實實驗數據，探討腫瘤微環境路徑之表現變化及觀察不同數據集之間於同一路徑下之相似/不同變化。
- 分析真實實驗數據集去建立與免疫相關分子(如：細胞激素)，並探討中間調控之生物功能。

更多詳細資料可參考：<http://tv.qiagenbioinformatics.com/video/66506029/investigating-the-tumor>

若有任何分析使用上的問題請洽 洪慈懋 產品專員 Office: 02-2795 1777 #3014 Mobile: 0970592091

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